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CUTANEOUS MALIGNANT MELANOMA – BODY SITE, SUN EXPOSURE, GENETIC FACTORS AND PROGNOSIS

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Cutaneous malignant melanoma – body site, sun exposure, genetic factors and prognosis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Science,

ABSTRACT

Background: The interplay between tumor site, ultraviolet radiation (UVR) exposure, genetic factors and various epidemiological parameters of cutaneous melanoma is complex. In this thesis, we aimed to investigate the impact of detailed body site of the primary tumor beyond the conventional and strictly anatomic division of the head-neck, trunk, upper- and lower extremities, focusing on body site division according to UVR exposure patterns in relation to risk, outcome and other factors.

Methods: We retrospectively reviewed medical records of patients with cutaneous melanoma to obtain detailed site information of the primary tumor (***studies I–IV***). We then entered detailed site on a three-dimensional anatomic model in a computer software, and classified body areas according to UVR exposure patterns, visibility upon skin self-examination, and anatomic classifications more detailed than the ICD (International Classification of Diseases) codes. Through linkage with regional and/or national registers, we obtained data on incidence and survival, as well as established confounders and (when applicable) losses to follow-up. We also reviewed medical records for sentinel node location and -status (***study I***), and performed PCR and pyrosequencing for mutation analysis of proto-oncogenes *BRAF* and *NRAS* (***study IV***).

Results: Trunk melanoma was associated with multiple but not uncommon sentinel node locations, compared to extremity melanoma. Multiple or uncommon sentinel node locations were not found to explain the association between trunk melanoma and reduced patient survival (***study I***). Melanomas assigned to intermittent UVR exposure patterns according to detailed anatomic site of the primary tumor displayed a higher incidence increase in the Stockholm-Gotland region during the past decades compared to melanomas on sites with assigned chronic UVR exposure patterns (***study II***). Site-assigned highly intermittent UVR patterns and poorly visible sites upon skin self-examination were associated with reduced patient survival compared to chronically UVR exposed and easily visible sites, respectively (***study III***). Intermittently UVR exposed sites were associated with *BRAF* mutations, and chronically UVR exposed sites with *NRAS* mutations (***study IV***).

Conclusions: Primary tumor sites with assigned intermittent UVR exposure patterns are presumably related to the incidence increase as well as reduced patient survival and genetic *BRAF* mutations of cutaneous melanoma, whereas sites assigned to chronic UVR exposure patterns contribute less to the incidence increase, are prognostically more favorable, and predominantly display *NRAS* mutations. Multiple or uncommon sentinel node locations do not explain the adverse prognosis of trunk melanoma.

Key words: Melanoma, Body site, Ultraviolet radiation exposure, Genetic factors, Incidence, Time trends, Sentinel node, Survival

LIST OF SCIENTIFIC PAPERS

I. **Sentinel node location in trunk and extremity melanomas: Uncommon or multiple lymph drainage does not affect survival**
Daniela Gordon, Karin E.Smedby, Inkeri Schultz, Henrik Olsson, Christian Ingvar, Johan Hansson, Peter Gillgren
Ann Surg Oncol. 2014 Oct;21(11):3386–94

II. **Time trends in incidence of cutaneous melanoma by detailed anatomical location and patterns of ultraviolet radiation exposure: a retrospective population-based study**
Daniela Gordon, Peter Gillgren, Sandra Eloranta, Henrik Olsson, Max Gordon, Johan Hansson, Karin E. Smedby
Melanoma Res. 2015 Aug;25(4):348–56

III. **Primary tumor sites in relation to ultraviolet radiation exposure and skin visibility correlate with survival in cutaneous melanoma**
Daniela Gordon, Johan Hansson, Sandra Eloranta, Max Gordon, Peter Gillgren, Karin E. Smedby
Int J Cancer. 2017 Oct;141(7):1345–54

IV. **Detailed anatomic site and patterns of ultraviolet radiation exposure among cutaneous melanoma patients in relation to *BRAF*/*NRAS* mutations**
Daniela Gordon, Henrik Green, Lena Kanter, Katarina Omholt, Suzanne Egyhazi, Peter Gillgren, Karin E. Smedby, Johan Hansson
Manuscript

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LIST OF ABBREVIATIONS

aHR	Adjusted hazard ratio
aOR	Adjusted odds ratio
aRR	Adjusted rate ratio
ALM	Acral lentiginous melanoma
BANS	Upper back, posterior arm, neck and scalp
BRAF	V-raf murine sarcoma oncogene homolog B1
BSA	Body surface area
C(M)M	Cutaneous (malignant) melanoma
CI	Confidence interval
DNA	Deoxyribonucleic acid
Essdoll©	Anatomical software (*see SkinTrac*©)
HR	Hazard ratio
ICD	International Classification of Diseases
IR	Incidence rate
IRR	Incidence rate ratio
IQR	Interquartile range
LDH	Lactate dehydrogenase
LMM	Lentigo maligna melanoma
LR	Likelihood ratio
NM	Nodular melanoma
OR	Odds ratio
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
PCR	Polymerase chain reaction
RMR	Regional Melanoma Register
RR	Relative risk
SES	Socioeconomic status
SkinTrac©	Anatomical software (*see EssDoll*©)
SMR	Swedish Melanoma Register
SN	Sentinel node

S(L)NB	Sentinel (lymph) node biopsy
SNL	Sentinel node location
SSE	Skin self-examination
SSCP	Single-strand conformation polymorphism
SSM	Superficial spreading melanoma
TANS	Thorax, upper arm, neck and scalp
UV(R)	Ultraviolet (radiation)

1 INTRODUCTION

The incidence of cutaneous melanoma in Sweden has increased fourfold from 1976 to 2015 (1). Ultraviolet radiation (UVR) exposure is the main risk factor of melanoma and changing UVR exposure patterns are thus regarded as drivers of the incidence increase. Intermittent UVR exposure is believed to contribute more than chronic UVR exposure. These exposure patterns have also been linked to different genetic mutations. Unfortunately, UVR exposures are difficult to quantify as each individual's exposure is a mix between intermittent and chronic UVR over a lifetime.

Scientific evidence is scarce on how different UVR exposure patterns cause and interact with other factors in cutaneous melanoma, and how a further incidence increase should be prevented. In fact, there are no signs of a decreasing melanoma incidence in Sweden despite repeated public health efforts to promote prevention, with an average annual percentage increase of about 5% from 2005 to 2015, and a mortality rate of 4† to 7‡ per 100,000 inhabitants (2015) (1). Hence, the societal costs of cutaneous melanoma are large, spanning from the suffering of patients and those close to them to economic costs of direct (healthcare) and indirect (productivity losses) nature.

Primary tumor site as classified in the International Classification of Diseases (ICD) is an independent prognostic factor for localized melanoma, and is often used as a proxy variable of UVR exposure patterns. However, it is a rather crude, and strictly anatomic, classification. A more detailed anatomic classification of primary body site may be of additional value regarding prognostic prediction. Also, a classification of the primary tumor site based on common skin coverage, or lack thereof, by clothes may better capture UVR exposure patterns.

In this thesis, we aimed to evaluate detailed body site of the primary tumor, mainly as a proxy variable for UVR exposure patterns but also from a purely anatomic perspective, in relation to (1) the incidence increase, (2) genetic factors, (3) sentinel node location and (4) prognosis of cutaneous melanoma.

† Women

‡ Men

2 BACKGROUND

2.1 INCIDENCE AND SURVIVAL

Incidence

From 1976 to 2015, the incidence (age-standardized to the Swedish population 2000 in parentheses) of melanoma has increased from 9.1 (9.4) to 39.3 (36.3) per 100,000 inhabitants for women and 8.6 (9.6) to 41.3 (41.6) for men (**Figure 2.1.1**) (1). Today, melanoma is the fifth and sixth most common malignancy in Sweden among women and men, respectively, with 3,951 new cases diagnosed in 2015, and a cumulative probability of 2.3–2.4% of developing melanoma before the age of 75 years (1).

Changing ***UVR exposure patterns*** have been suggested as the main driver of this increase, although early diagnosis and a diagnostic drift (2), where borderline lesions are increasingly classified as malignant, cannot be excluded as contributory factors.

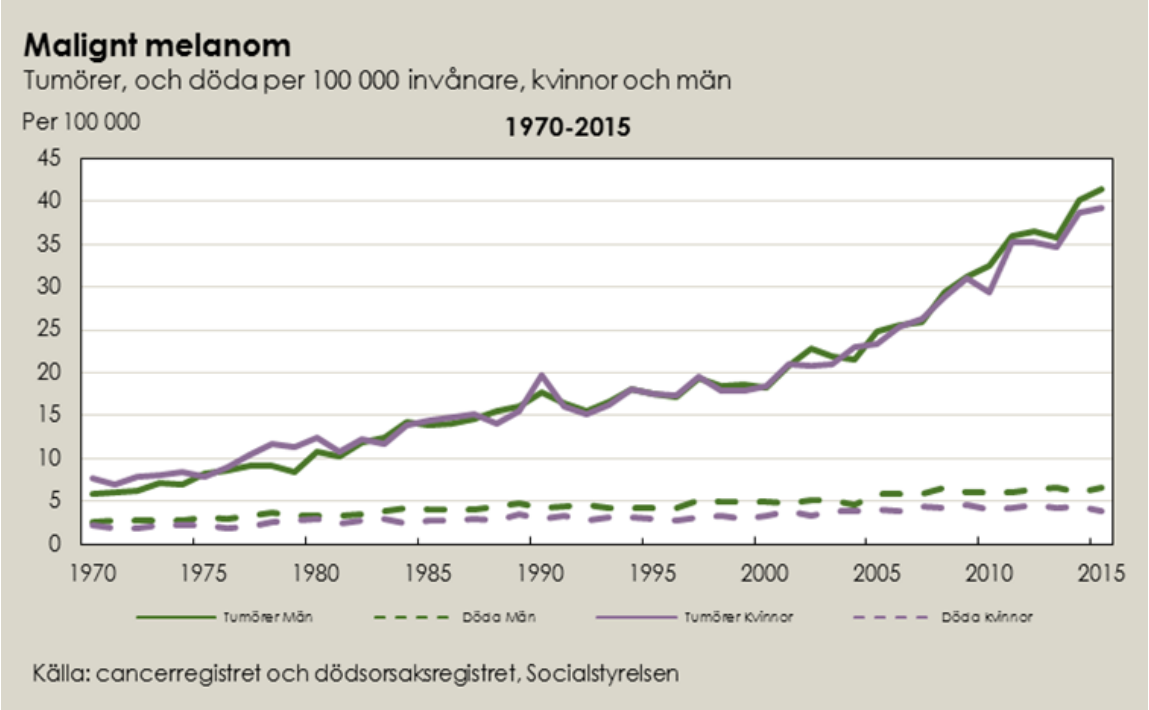


Figure 2.1.1 Incidence (number of tumors per 100,000 inhabitants) and mortality of malignant melanoma in Sweden by sex (1). Compiled from data from the Swedish Cancer Register and Cause-of-Death Register.

Aims have been set to reduce the incidence to levels below that of the year 2000 by 2020 (3), but despite primary prevention efforts there are no indications of a stabilization. In fact, a comparison showed continued increases in all age groups from 25–30 years of age and older,

from 1993–1995 to 2013–2015 (**Figures 2.1.2–2.1.3**) (1)**.**

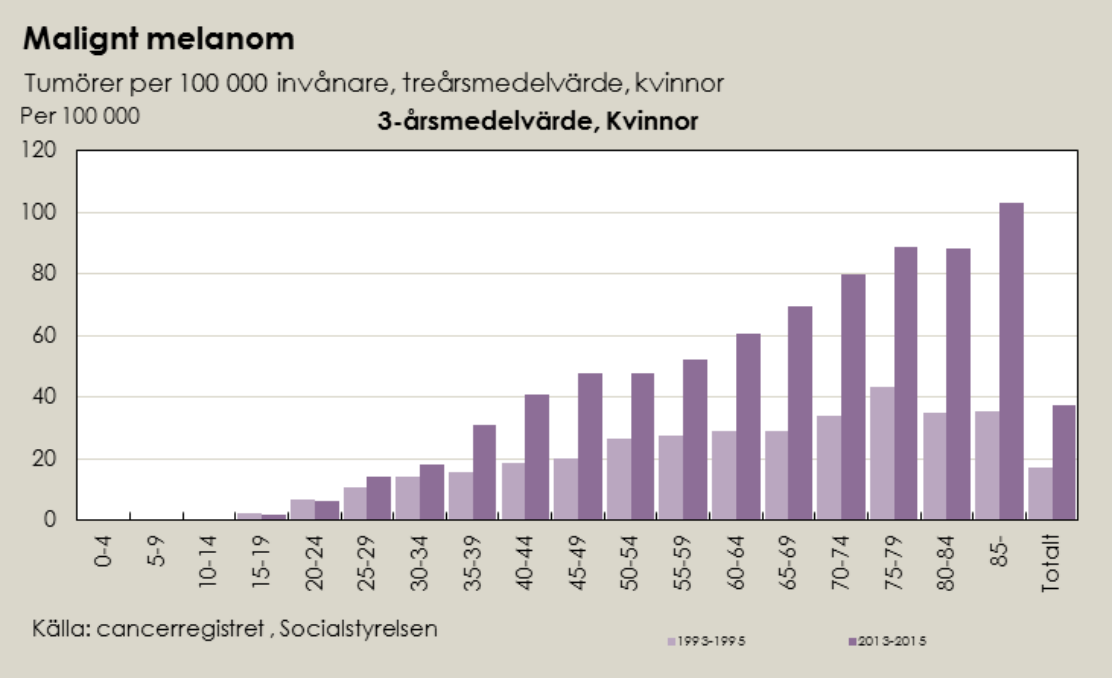


Figure 2.1.2. Incidence (number of tumors per 100,000 inhabitants) of malignant melanoma in Sweden among women (1). Compiled from data from the Swedish Cancer Register.

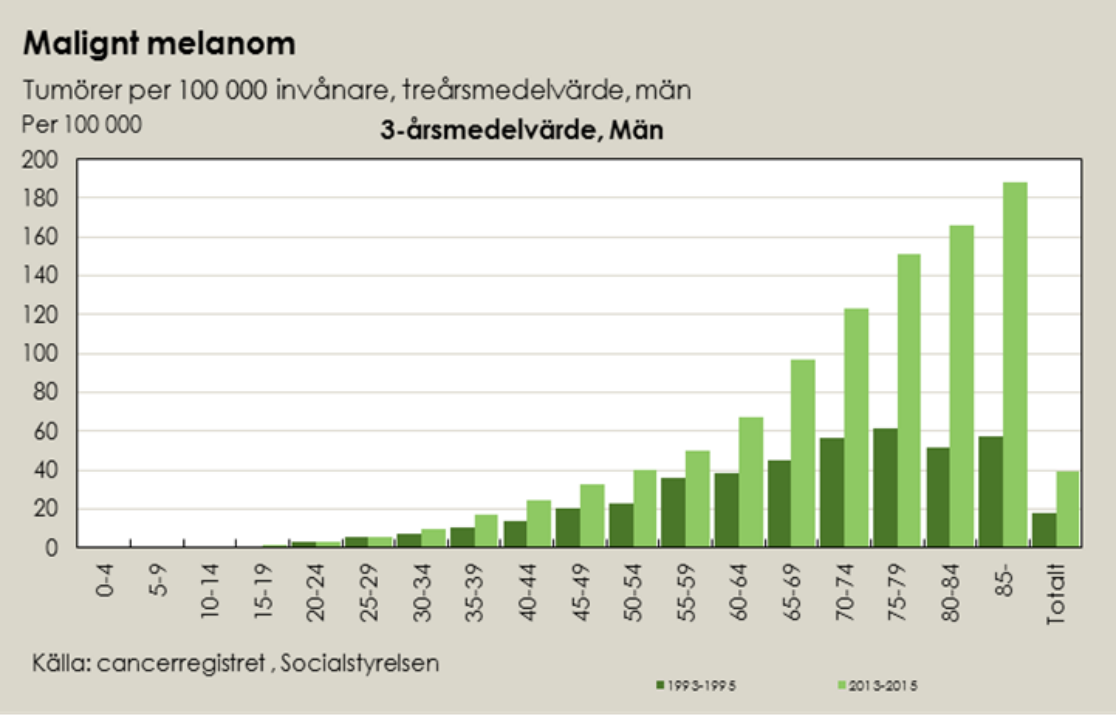


Figure 2.1.3. Incidence (number of tumors per 100,000 inhabitants) of malignant melanoma in Sweden among men (1). Compiled from data from the Swedish Cancer Register.

Survival

Mortality rates in Sweden due to melanoma have been more stable from 1976 to 2015 compared to the incidence increase, but the trend is still that of an increasing mortality in the population, with a rate of 2.0 deaths per 100,000 inhabitants for women and 2.9 for men in 1976, and 3.9 and 6.6. in 2015, respectively (1). However, between 2012 and 2015 the death rates have remained largely unchanged (**Figure 2.1.1**). Whereas increasing age at diagnosis and increased numbers of thick lesions may be contributory factors to the long-term mortality trend (4), it will be interesting to follow the continued development of mortality rates in the context of the new oncologic therapies introduced for stage IV melanoma during the past years.

The likelihood of survival is high among individuals with stage I melanoma (*see section 2.4.1 for stage definition*s), and low among those with stage IV melanoma, with a 5-year survival of <20% among the latter (**Figures 2.1.4–2.1.5**) (5). Among young individuals, death due to melanoma constitutes 8% of all tumor-related deaths (6).

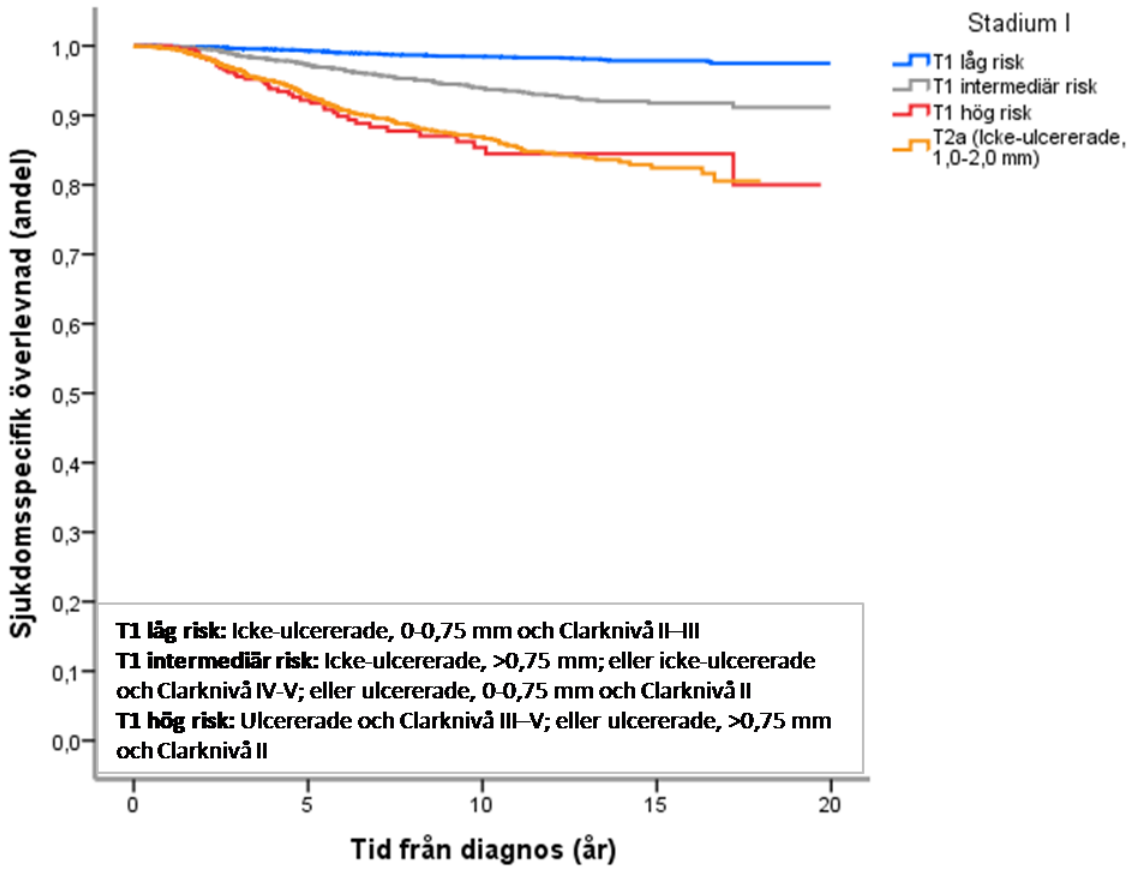


Figure 2.1.4. Melanoma-specific survival proportion over time (years) among patients with stage I melanoma (5). From the Swedish Melanoma Register April 2012.

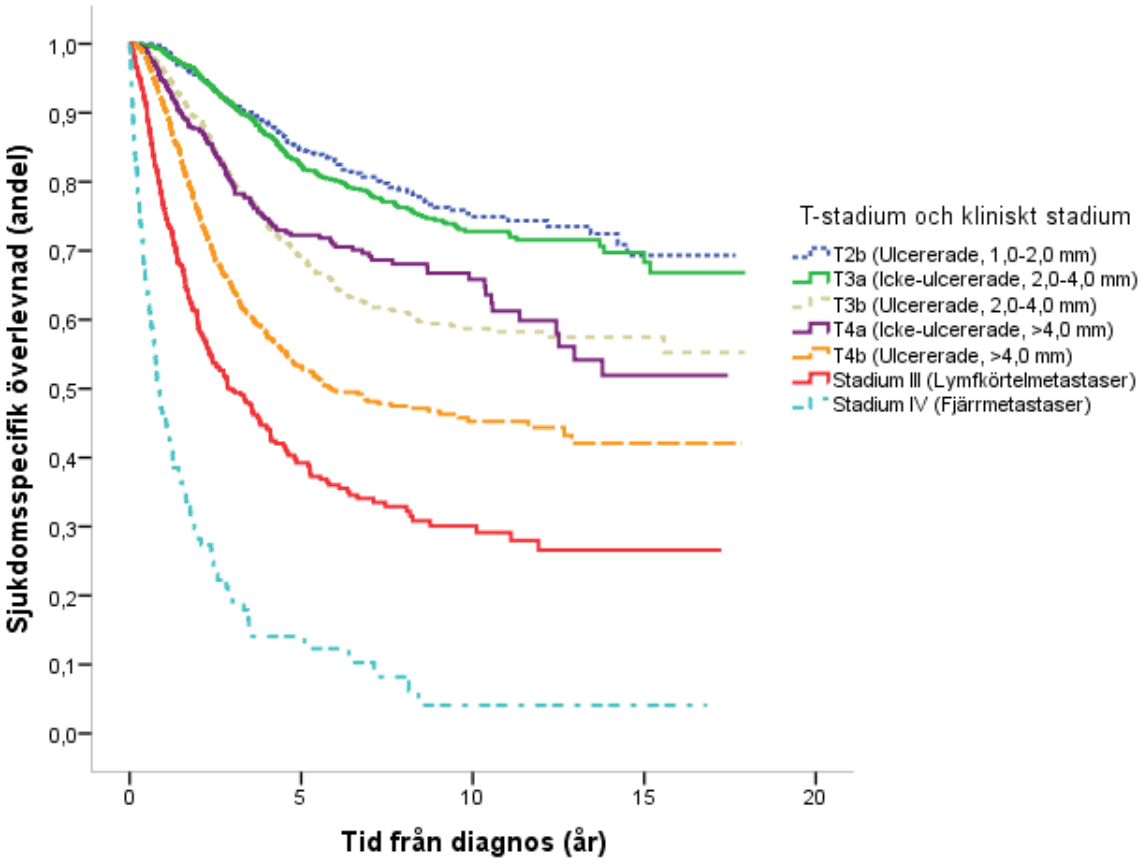


Figure 2.1.5. Melanoma-specific survival proportion over time (years) among patients with clinical stage II–IV melanoma (5). From the Swedish Melanoma Register April 2012.

2.2 RISK FACTORS

2.2.1 Environmental

Ultraviolet radiation (UVR) exposure is necessary for life on Earth and for our physical health and psychological well-being. At the same time, UVR radiation is also a ***class I carcinogen***, with a potential to cause cancer comparable to the use of tobacco, and the main environmental risk factor for cutaneous melanoma (7,8). The statement specifically concerns i) solar radiation, ii) UVR including UVA, UVB and UVC, and iii) UV-emitting devices.

Solar radiation and UVR

Of the UVR emitted from the sun, UVC (wavelength 100–280 nm) does not penetrate the ozone layer of the stratosphere, whereas UVA (>315–400 nm) constitutes the larger part (95%) of UVR that reaches the earth, and UVB (>280–315 nm) a lesser part (5%) (8,9).

The intensity of UVR exposure is determined by the ***time of the day***, ***season***, ***latitude***, ***altitude***, ***surface*** reflection, ***cloud*** formation, the ***ozone*** layer, and degree of ***pollution*** (8). The most intense exposure is emitted between 11:00 and 15:00 (10).

UV intensity can be quantified using the **UV index** (11). It is determined by the maximum UVR intensity during a 24-hour period, and varies significantly by latitude, season and time of the day as well as weather conditions and the ozone layer. Levels 1–2 are considered low, 3–5 moderate, 6–7 high, 8–10 very high, and ≥11 extreme. In brief, low levels are generally considered safe with regard to outdoor activities, whereas higher levels warrant increasing sun protective measures. In Sweden, the UV index in the summer is moderate to high in clear weather, whereas in Southern Europe levels are very high, and in Thailand extreme (12).

Tanning devices

The most commonly used tanning devices among the public almost exclusively emit UVA (>>95%), but also a small amount of UVB (<<5%) (8,13). Exposure to artificial UVR from sunbeds at a young age (< 35 years) has been clearly associated with the development of cutaneous melanoma (HR 1.75; 95% CI 1.34–2.26) in a large meta-analysis (13). Further, evidence does not favor prophylactic use of these devices in order to reduce sun-induced damage from later solar radiation (14).

UVR exposure patterns

Although the relationship between UVR exposure and melanoma development is complex, two main patterns have been proposed to describe UVR exposure: intermittent and chronic UVR exposure patterns.

Comparison of studies on **intermittent UVR exposure** is complicated by the varying definitions and methods to quantify this exposure, but cumulative evidence supports the

importance of intermittent UVR exposure as a risk factor for cutaneous melanoma (15–25). Intermittent UVR exposure is often referred to with regard to situations when skin without recent exposure is exposed to intense UVR exposure and thus prone to ***sunburn***, which has also been linked to melanoma development (19–24,26–34). Examples of intermittent UVR exposure behavior in the literature include vacations on sunny latitudes, active sunbathing, beach sports, use of sunbeds and previous sunburns. Further, primary tumor sites on the trunk or extremities have been used as proxy variables of intermittent UVR exposure (*see section 2.2.2*).

Chronic UVR exposure has instead been used with reference to more long-term or continuous exposures that are related to the development of melanization and a protective ***tan***, and ***cumulative sun-induced damage***. Examples include outdoor occupational UVR exposure, primary tumor site on the head and neck (*see section 2.2.2*), and signs of cumulative sun-induced damage (marked/severe elastosis). The evidence with regard to chronic UVR exposure and the risk of developing melanoma is less clear than that of intermittent UVR exposure, but points toward a neutral (23) or even protective (24,25,35) association.

Genetic mutations

Genetic **"fingerprint"** or **signature mutations** such as cyclobutane pyrimidine dimer formation have been associated with UVR exposure (9,13). Interestingly, the ***BRAF*** and ***NRAS*** mutations commonly found in cutaneous melanomas do not display this fingerprint pattern. However, a linkage to UVR exposure is indicated by other UV-related mechanisms (36), the distribution of these mutations by primary tumor site (*see section 2.2.2*), and the absence (37–40) or presence (38) of signs of cumulative sun-induced damage (38), respectively. *BRAF* mutations have also been associated with sunburn in animal experiments (41).

2.2.2 Constitutional

Genotype, phenotype and family

The most commonly mutated gene in hereditary melanoma, ***CDKN2A***, entails a very high risk (60-fold risk increase) of developing cutaneous melanoma among family members that are mutation carriers (42). Individuals belonging to families with **familial melanoma** without mutated *CDKN2A* are also at increased risk of developing melanoma, as are those that do not classify into the latter category but have (at least) one **first-degree relative** diagnosed with melanoma (42,43).

A third category of risk individuals are those with sensitive **pigmentation traits** with regard to skin color and skin type according to Fitzpatrick, hair and eye color (43), and a high number (44) (>50–100) and/or large size (>5 mm) of **melanocytic nevi** (42). ***MC1R*** alterations are common among individuals with a fair skin color that burns easily and tans

poorly, and red hair, but the gene may also contribute to the risk of melanoma through pigment-independent pathways (45).

Sex and age

Melanoma is very uncommon until puberty, and increases progressively thereafter (*see Figures 2.1.2–2.1.3*) (1), with a median **age at diagnosis** of 64 years for men, and 60 for women (4). However, UVR exposure during childhood is a risk factor for cutaneous melanoma (32,46), supporting the concept of a latency phase between exposure and disease development. It has also been demonstrated that UVR exposure in childhood is associated with the development of nevi (47), and that the distribution of nevi among children correlates with the site distribution of melanoma among young adults (48).

Anatomic site

The most common cutaneous melanoma site according to the ICD code is the trunk among men, and the lower extremities among women. Meanwhile, melanoma on head and neck sites is mainly related to older age (**Figure 2.2.2**) (5,49). Melanomas on the trunk are often associated with nevi (50–54). Melanomas of unknown, and non-cutaneous (mucosal and ocular) body sites are not covered in this thesis.

The incidence increase of the past decades has occurred mainly for trunk and extremity melanomas, whereas melanomas on the head and neck have increased less in most reports (55–61). Further, upper extremity melanomas have increased among men during recent years (4).

Study-specific aspects

Given that UVR exposure is the main risk factor for sporadic cutaneous melanoma, it is believed that the increased incidence on different body sites reflects changes in UVR exposure behavior among the public. Specifically, trunk and extremity sites are often used as crude proxy variables for ***intermittent UVR exposure***, and head and neck sites for ***chronic UVR exposure***. However, no previous studies have been designed to specifically evaluate the association between detailed anatomic site classified by intermittent vs. chronic UVR exposure in relation to ***incidence trends*** using an established UVR exposure model ***(→study II***).

In a similar fashion, the preponderance of *BRAF* mutations among trunk and extremity melanomas (37,62–65) and sites without signs of cumulative sun-induced damage (37–40), and *NRAS* on sites with signs of cumulative sun-induced damage (38), has suggested that ***BRAF*** mutations may be associated with ***intermittent UVR exposure***, and ***NRAS*** mutations with ***chronic UVR exposure***, but the hypothesis has not been evaluated in a more detailed UVR exposure model ***(→study IV***).

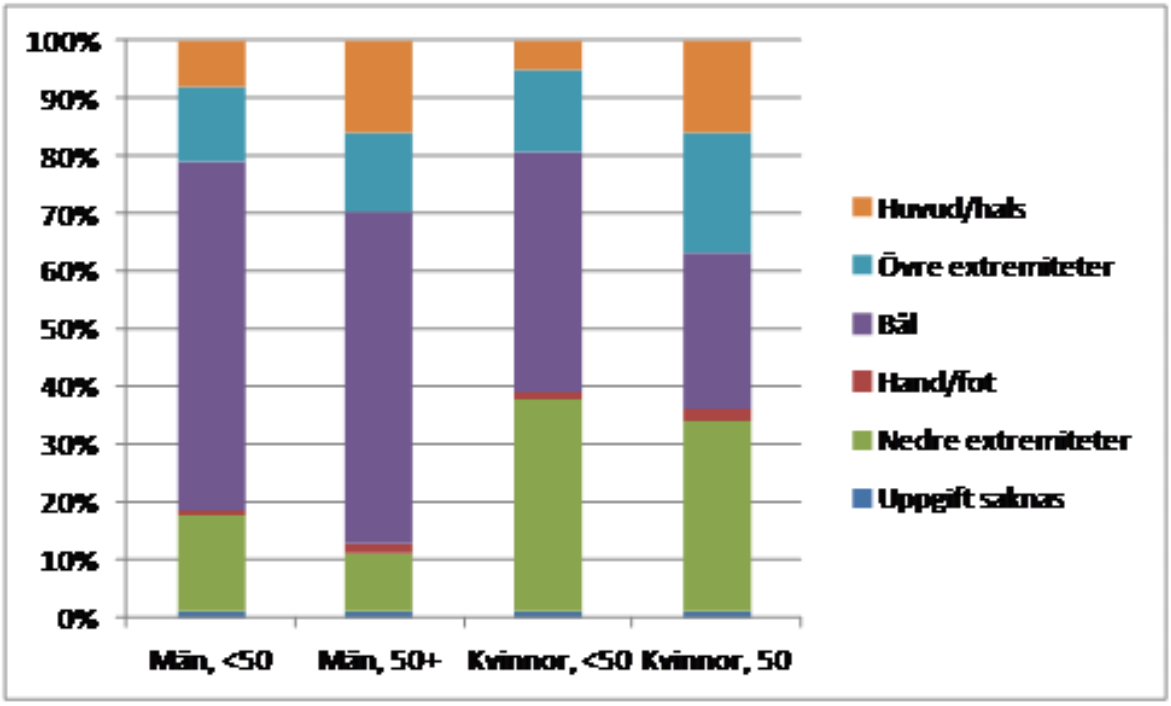


Figure 2.2.1. Body site, sex and age of patients diagnosed with cutaneous melanoma 1990−2008 (5). From the Swedish Melanoma Register 2012-03-15.

2.2.3 Psychology/lifestyle

Psychology

The psychological driving components of UVR exposure include **well-being** and **esthetic incentives** (6). A recent study from 2015 among Swedish high-school students showed that their sun exposure behavior is highly affected by esthetic ideals favoring a tanned complexion (6). Of particular concern is that 18–32% reported that a "good tan" is desirable even if it entails a slight sunburn. This coheres with findings from international studies, where a strong "**tan-seeking behavior**" (66), poor sun-protective behavior and more frequent sunburn episodes (67) have been observed in Sweden compared to other countries.

Lifestyle

During the past decades, **travel** to sunny destinations has become increasingly available to the public. This increase is demonstrated below in a graph compiled from flight data from the Swedish Aviation Authority (**Figure 2.2.3**) (68).

Although causality cannot be determined between flight data and UVR exposure/melanoma development, it is feasible to hypothesize that increased charter travel to sunny destinations and the related sudden change of latitude and high-intensity intermittent UVR exposure is associated with the increased incidence of melanoma during the past decades. The identification of post-secondary **education** (compared to compulsory education only) as a

risk factors for melanoma (1) may also cohere with this hypothesis.

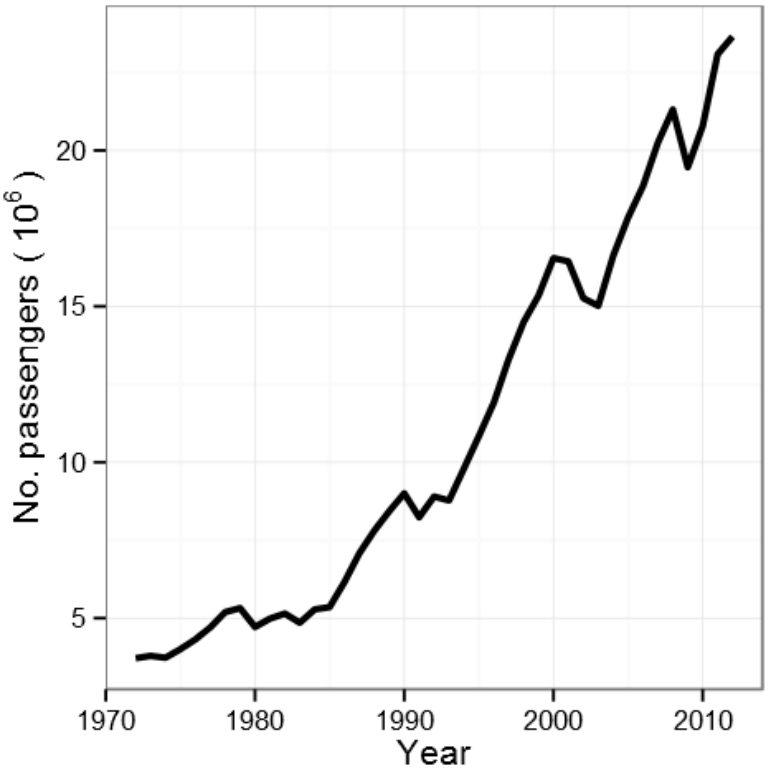


Figure 2.2.3. Number of arriving and departing passengers in scheduled and non-scheduled international traffic at Swedish airports 1972 to 2012. Compiled from data from the Swedish Aviation Authority (68).

2.2.4 Other risk factors

Other risk factors for developing cutaneous melanoma include a history of **previous melanoma** or **other skin cancer** (42).

2.3 PROTECTIVE FACTORS

Shadow, **clothing**, and the use of **sunscreen** (69) are regarded as protective factors with regard to the risk of developing cutaneous melanoma. However, the use of sunscreen can prolong UVR exposure (70,71), which may at least partially explain the results of studies showing a lack of protective, or even adverse, associations between the use of sunscreen and melanoma.

2.4 PROGNOSTIC FACTORS

2.4.1 Stage and TNM classification

The likelihood of melanoma survival depends largely on **stage** and **TNM classification**, with great differences between these categories (*see Figures 2.1.4–2.1.5*) (5). Stage and TNM category can be determined on a clinical (**Table 2.4.1**) or histopathological basis, of which the latter includes a subdivision of stage III melanomas into the stages IIIA, B and C based on a detailed classification of lymph nodes in the N category (N1a–N3) obtained through sentinel node biopsy or lymph node dissection (72,73).

For macroscopically localized melanoma (*i.e.* no evidence of metastases upon clinical or radiological evaluation), tumor thickness, ulceration, mitotic rate and sentinel node status (74) are strong independent prognostic factors. Of these, sentinel lymph node status is the strongest one, since it identifies patients with and without microscopic loco-regional spread.

Table 2.4.1. Clinical stage and TNM classification (72,73).

Clinical stage	**T**	***Thickness (mm)***	***Ulceration***	***Mitoses (n / mm^2)***	**N**	**M**
0	Tis	n/a	n/a	n/a	N0	M0
IA	T1a	≤1.0	no*	0*	N0	M0
IB	T1b	≤1.0	yes*	≥1*	N0	M0
	T2a	1.01–2.0	no	n/a	N0	M0
IIA	T2b	1.01–2.0	yes	n/a	N0	M0
	T3a	1.01–2.0	no	n/a	N0	M0
IIB	T3b	1.01–2.0	yes	n/a	N0	M0
	T4a	1.01–2.0	no	n/a	N0	M0
IIC	T4b	1.01–2.0	yes	n/a	N0	M0
III	T†	n/a	n/a	n/a	N>0	M0
IV	T†	n/a	n/a	n/a	N‡	M1

† All T categories.

‡ All N categories.

* T1a and b classification based on the presence or absence of ulceration *or* mitoses.

T ─ Tumor

The T category is determined by ***tumor thickness***, ***ulceration*** and ***mitotic rate***.

N ─ Nodal

The N category is determined by the number of **metastatic lymph glands** and the **metastatic burden** as defined by the presence of macroscopic, microscopic or satellite/in-transit metastases. Microscopic metastases are evaluated using **sentinel lymph node biopsy** (SLNB) [A] (75). In addition to being a diagnostic tool, procedure-related melanoma-specific survival benefits (compared to nodal observation) have been shown among intermediate thickness (1.2–3.5 mm) melanomas (76). Individuals with nodal metastases are classified as having ***stage III melanoma***.

[A] SLNB is a staging procedure for clinical stage IB and II, and selected cases of high-risk stage IA, melanoma. The procedure is based on the postulation that the first lymphatic node(s) that drains lymphatic fluid from the primary tumor is a gate-keeper (***sentinel***) of metastatic spread, *i.e.* the absence of tumor cells in a sentinel lymph node indicates localized disease whereas the presence of tumor cells indicates loco-regional and possible further spread.

In brief, a radiocolloid and blue dye are injected around the primary tumor and followed to one or several lymph nodes in the first adjacent regional lymph node basin or (less commonly) other node locations, where the node(s) are removed and undergo histopathological evaluation. Based on the latter, **sentinel node status** is determined as ***positive*** (at least one pathological lymph node) or ***negative*** (disease-free lymph nodes only).

M ─ Metastasis

The M category is determined by the presence or absence of distant metastases. Individuals with distant metastases (M1) are classified as having ***stage IV melanoma***. M1 melanomas can be subdivided into the categories M1a–c based on the **metastatic site** and lactate dehydrogenase (**LDH**) levels.

2.4.2 Other prognostic factors

Patient factors

Prognostic patient factors include sex (77–82), age (78–81), socioeconomic status (83), cohabitation (84), and comorbidities (85).

Anatomic site

Anatomic site of the primary tumor according to the ICD classification is an independent prognostic factor (86–88), with a worse prognosis for the trunk (82,86,87) and scalp (89–91) compared to the face and extremities. The mechanism behind this is unknown. Further, evidence is conflicting with regard to the importance of detailed anatomic site division in relation to patient survival.

Study-specific aspects

Since melanomas on trunk sites display lymphatic drainage to uncommon (*see section 4.4 for definition*) (92–105) and multiple (106–112) ***sentinel node locations*** more often than those on extremity sites, it has been hypothesized that these could be related the reduced survival among individuals with trunk melanoma (86,113–116). However, previous studies have suffered from limited power or evaluated short-term prognostic outcomes such as sentinel node status or recurrence, and results are inconsistent (→***study I***).

Further, the impact of a more ***detailed anatomic site*** division than the ICD code on patient survival is not clear, given the small size of most of these studies, and differences in inclusion and adjustment for confounders (*see section 4.5*) (→***studies I*** and ***III***).

The impact of detailed anatomic site classified by ***intermittent*** and ***chronic UVR exposure*** has to our knowledge not been previously studied with regard to patient survival (→***study III***).

3 AIMS AND HYPOTHESES

Overall aim

The overall aim of this thesis was to investigate the role of detailed anatomic tumor site a in relation to sentinel node location, ultraviolet radiation (UVR) exposure patterns, genetic factors and prognosis of cutaneous melanoma.

Specific hypotheses

Specifically, we wanted to test the following hypotheses:

1. The lower survival among patients with trunk melanoma (compared to extremity melanoma) is associated with uncommon and/or multiple sentinel node locations.
2. Intermittent UVR exposure patterns, assigned by detailed tumor site, drive the incidence increase of cutaneous melanoma compared to chronic UVR exposure patterns.

3a. Site-assigned intermittent UVR exposure is a negative prognostic factor compared to chronic UVR exposure.

3b. Visibility upon skin self-examination is related to site-assigned UVR exposure and prognosis.

3c. Subdivision of tumor site beyond the four major sites (head-neck, trunk, arms and legs) commonly used in survival analyses improves the accuracy of prognostic prediction.

4. Site-assigned intermittent and chronic UVR exposure is associated with *BRAF* and *NRAS* genetic mutations, respectively.

4 METHODOLOGICAL CONSIDERATIONS

4.1 SETTING

The healthcare system in Sweden is publicly funded and every citizen is entitled to equal care within this system. It is organized into six regions, of which the Stockholm-Gotland healthcare region with about 2.3 million inhabitants (23% of the total population) (117) is the largest one with regard to population size, and the study base for ***studies II-IV***. For ***study I,*** the Stockholm-Gotland healthcare region is part of the study base, together with the Western (1.9 million inhabitants) and Southern (1.8 million inhabitants) healthcare regions.

4.2 STUDY DESIGN AND POPULATION

The **study design** is a key element that defines a study's ability to answer research questions. All studies in this thesis are retrospective cohort studies making use of prospectively collected register data. A retrospective cohort study is an **observational study**, *i.e.* the exposure of interest is not randomized or controlled, but merely observed, by the researchers of the study. This type of study can determine whether two factors (A and B) are associated to one another, but not whether factor A causes factor B. The **cohort** design means that individuals that differ regarding an exposure are followed over time and compared in relation to one or several outcomes.

The population sample that is studied is called the **study population**. A **population-based** study means that the study sample is representative of the **source population** in the sense that all individuals of the source population that fulfill certain criteria are included.

Study-specific aspects

Study I is a population-based multicenter cohort. We included individuals diagnosed with primary invasive localized trunk or extremity melanoma who had undergone lymphoscintigraphy in three (Stockholm-Gotland, Western and Southern) healthcare regions from January 2000 to December 2006 (Sahlgrenska University Hospital) or 2008 (Karolinska University Hospital Solna, Södersjukhuset, Skåne University Hospital Lund). The individuals were identified from hospital registers. Since the procedure was introduced in Sweden during this period, eligibility criteria for the procedure were not static, but in brief consisted of invasive melanoma with a thickness of >1−1.5 mm, or <1 mm if other adverse characteristics were present. Individuals with traceable records in the Swedish Melanoma Register (SMR) and medical records that had undergone lymphoscintigraphy for a single invasive melanoma within six months of diagnosis (n = 859) remained in the study, whereas others (n = 69) were excluded.

Study II is a population-based cohort study from the Stockholm-Gotland healthcare region. The inclusion was register-based, with five bi-annual cohorts (1977−1978, 1983−1984, 1989−1990, 1995−1996 and 2000−2001) of individuals diagnosed with invasive or *in situ* melanoma from the Regional Melanoma Register (RMR) (118) assessed for eligibility. To enable evaluation of detailed site of the primary tumor, we excluded individuals for whom the site of the primary tumor was unknown at diagnosis (n = 95). If an individual was diagnosed with more than one melanoma during the study period, the first was kept in the study whereas subsequent tumors were excluded. The final cohort consisted of 3,058 individuals.

Study III is the third population-based study of the thesis and included all individuals diagnosed with invasive melanoma from 1976 to 2003 registered in the RMR. We excluded individuals with metastatic disease at diagnosis, more than one invasive melanoma, or missing data on detailed anatomic site of the primary tumor or the variables above (*see original paper for details*). In case of multiple tumors of which only one invasive lesion, the patient and the invasive lesion was kept in the study, whereas the *in situ* lesions were excluded. After exclusions, there were 5,973 individuals left to analyze.

Study IV consists of one new and one previously studied cohort of individuals diagnosed with melanoma. The new cohort included 148 individuals with primary tumors located on the face or ears, diagnosed from 1996 to 2009 with representative samples available at the Department of Pathology at the Karolinska University Hospital Solna and a record in the RMR.

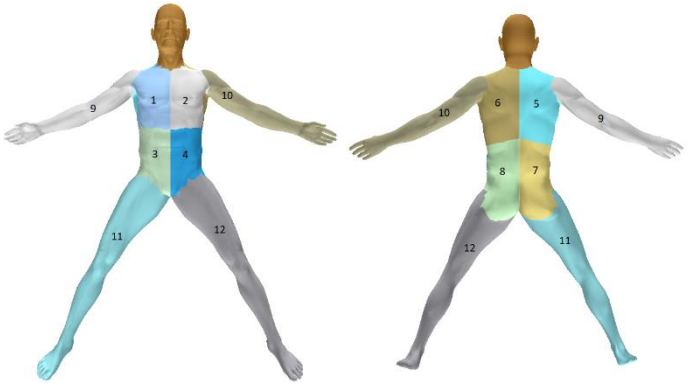
The previously studied cohort included primary and/or metastatic melanomas with known mutation status for *NRAS* and *BRAF*, analyzed at the same department. The primary site of these melanomas was mostly trunk or extremities, and the year of diagnosis ranged from 1977 to 2002. From this cohort, we excluded previously performed mutation analyses based on metastatic tissue only in the presence of multiple primary tumors or a primary tumor of unknown site (*i.e*. no reliable connection could be made to a single primary tumor site), and those without a primary tumor record in the RMR, leaving 183 individuals with 185 melanomas for analysis. If both primary and metastatic tissue had been analyzed, we used the results of the primary tissue analyses. Together, the new and previously studied cohort included 331 individuals with 333 melanomas.

4.3 EXPOSURES

Exposure is a term used in epidemiology to define a factor that may (or may not) be associated with one or several outcome(s).

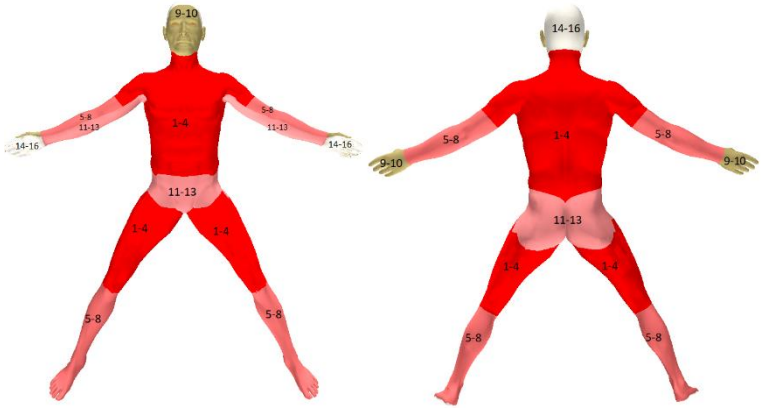
In all studies (***I-IV)***, the main exposure was **detailed anatomic body site** of primary cutaneous melanoma.

4.3.1 Quadrant model



In ***study I***, we first classified the body surface into two major anatomic areas: trunk and extremities. To enable more detailed analysis, we then divided the ***trunk*** further into 8 subsites: right (no. 1) and left (no. 2) upper anterior, right (no. 3) and left (no. 4) lower anterior, right (no. 5) and left (no. 6) upper posterior, and right (no. 7) and left (no. 8) lower posterior quadrant. The ***extremities*** were divided into right (no. 9) and left (no. 10) upper, and right (no. 11) and left (no. 12) lower extremity.

4.3.2 UVR exposure model



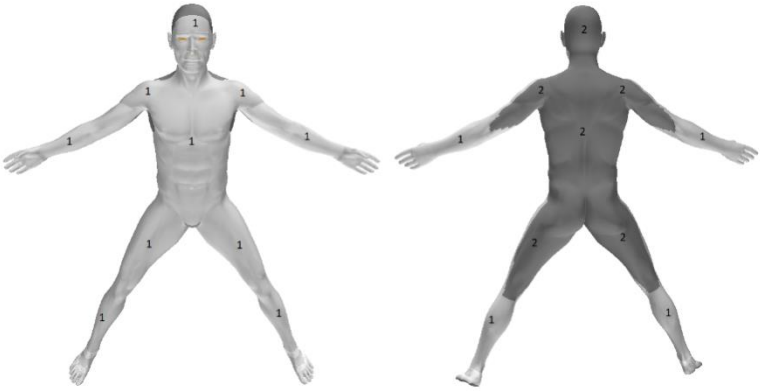
Studies II-IV are based on a UVR classification model developed by *Augustsson et al* (119), that first appeared in scientific papers in the early 1990s. The Augustsson model was designed to classify the body surface according to presumed clothing and sun exposure habits. The full model consists of 4 major anatomic sites, divided into 16 subsites: ***intermittent*** (no. 1−8), ***chronic*** (no. 9−10), ***rare*** (no. 11−13) and ***other*** (no. 14−16). The subareas are chest (no. 1), back (no. 2), anterior (no. 3) and posterior (no. 4) thighs, lateral arms (no. 5), anterior (no. 6) and posterior (no. 7) lower legs, dorsum of feet (no. 8), face (no. 9), dorsum of hands (no. 10), medial arms (no. 11), lower abdomen (no. 12), buttocks (no. 13), scalp (no. 14), palms (no. 15), and soles (no. 16).

In ***studies II−III***, we modified the Augustsson model slightly and divided the intermittent area further into ***highly intermittent*** (no. 1−4) and ***moderately intermittent*** (no. 5−8) sites (***paper IV***), or ***intermittent (core)*** and ***intermittent (peripheral)*** sites as they were formerly referred to (***paper III)***. The reason for this was an empirical observation/hypothesis that the central (core) parts of the intermittent category (chest, back and thighs) are exposed to the sun mainly during active sun bathing or summer sport activities, whereas the peripheral parts (lateral arms, lower legs and dorsum of feet) display a more continuous seasonal exposure.

The relative body surface areas of the original two-dimensional model by *Augustsson et al* were estimated using a slightly adapted Lund & Browder method (120). The body surface

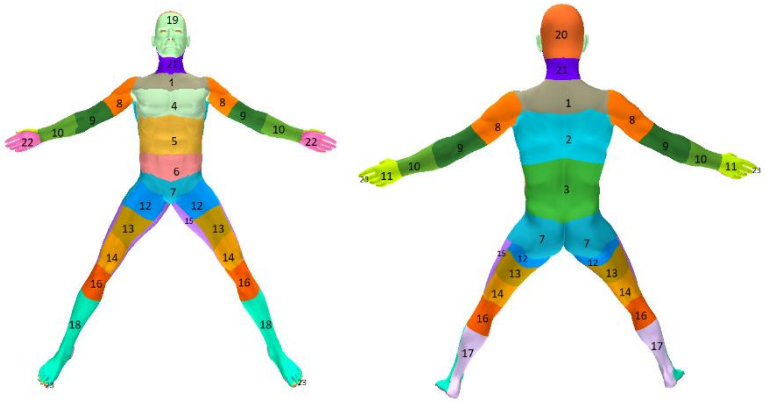
areas of the three-dimensional model created in SkinTrac© (*see section 4.6*) were similarly calculated by the computer software in relation to the whole-body surface.

4.3.3 Skin visibility model



In ***study III***, we also classified the body surface according to presumed visibility upon skin self-examination, and major anatomic sites and subsites. In the empirical visibility classification, skin sites were divided into easily or poorly visible sites. ***Easily visible sites*** include the face, anterior ears, chest, abdomen, anterior upper arms and thighs, lower arms and legs, dorsum of hands and feet, and palms (no. 1), whereas ***poorly visible sites*** consist of the scalp, retroauricular area, posterior upper arms and thighs, buttocks, pubic area and soles (no. 2).

4.3.4 Anatomic subsite model



For the classification of anatomic subsites, we used a classification by *Gillgren et al* (116) from a previous article by our group. However, in this study we performed minor adjustments where the number of subsites were reduced to 23. We also merged the subareas into five major anatomic sites; ***trunk*** (no. 1−7), ***arms*** (no. 8−11), ***legs*** (no. 12−18), ***head-neck*** (no. 19−21) and ***acral*** (no. 22−23). Subareas include the dorsal shoulders, superior back and clavicular area (no. 1), scapular and subscapular area (no. 2), middle and lower back (no. 3), supramammary and mammary area (no. 4), superior and middle abdominal area (no. 5), inferior abdominal area (no. 6), buttocks, pubic, genital and inguinal area and lateral hips (no. 7), upper (no. 8), middle (no. 9) and distal (no. 10) arms, dorsum of hands (no. 11), upper (no. 12), middle (no. 13) and distal (no. 14) thighs, medial and lateral thighs (no. 15), knee and popliteal area (no. 16), calves and achilleas area (no. 17), anterior lower legs and dorsum of feet (no. 18), face and ears (no. 19), scalp (no. 20), neck (no. 21), palms and soles (no. 22), and combined subungual area (no. 23).

4.4 OUTCOMES

Outcome is a term used to define a factor that may (or may not) be linked with one or several causal factor(s), *i.e.* exposure(s).

Patient survival

In ***studies I*** and ***III***, the main outcome was patient survival, defined as time from melanoma diagnosis to death. The main survival outcome measure was ***melanoma-specific survival***, complemented by calculations of ***all-cause survival***. Melanoma-specific death was determined by the main underlying cause of death in the cause of death certificate. In ***study I***, the study endpoint was December 31st, 2011, and in ***study II*** December 31st, 2013.

Individuals who were ***alive*** at the study endpoint, ***emigrated*** or ***died from other causes*** than melanoma during the follow-up period were censored in the main analyses. The term censored means that these individuals had not experienced the event of interest (in this case death) when last followed (the date of censoring), but information thereafter is missing. The time from diagnosis to the date of censoring is included in calculations as time at risk for the event of interest.

Sentinel node locations

In ***study I***, we also evaluated the presence of uncommon, multiple, contralateral and inaccessible sentinel node locations. ***Uncommon*** sentinel node locations were defined as all non-regional (*i.e.* non-axillary, non-groin) locations, and classified by anatomic structures into epitrochlear, cervical, supra- or infraclavicular, intrathoracal or -abdominal, popliteal or interval/in-transit (*i.e.* located along the lymphatic system between the primary tumor and a lymph node basin) nodes. An individual with at least one uncommon node was classified into this category. Patients with more than one node location were defined as having ***multiple*** node locations, and those with at least one node on the opposite side of the body in relation to the primary tumor as having ***contralateral*** node location. ***Inaccessible*** nodes were defined as nodes that were not accessible for surgery according to the surgeon, or predetermined as accessible but not found upon surgery. Patients who had undergone lymphoscintigraphy with inconclusive results were also included in this category. Like above, individuals with at least one inaccessible node were classified into this category.

Time trends

In ***study II***, the main outcome was time trends of overall ***melanoma incidence***. As a secondary outcome, we evaluated incidence per body surface area (BSA). The time periods investigated were 1977−1978, 1983−1984, 1989−1990, 1995−1996 and 2000−2001. Individual-level data was obtained for individuals diagnosed with melanoma, whereas for the underlying (Stockholm-Gotland) population, aggregated end of year estimates were obtained by calendar year, 1-year age groups and sex.

Mutation status

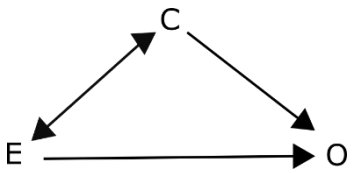
In ***study IV***, the main outcome was ***NRAS*** (Neuroblastoma RAS viral (v-ras) oncogene homolog) and ***BRAF*** (V-raf murine sarcoma oncogene homolog B1) mutation status. For both genes, mutation status was defined as either mutated or wild type. For mutated samples, the specific mutated genotype was recorded.

4.5 CONFOUNDERS AND INTERACTION VARIABLES

The classic explanation of a **confounder** [A] is a factor that is independently related to (but not an intermediate of) a studied exposure and outcome and, as a result, may create false associations (***type I error***) or eliminate true associations (***type II error***) between the exposure and outcome. In studies of cause-specific associations, it is therefore vital to adjust for established as well as potential confounders.

[A]

C = confounder
E = exposure
O = outcome



In melanoma studies, confounders can be divided into patient- and tumor-related factors. Sex and age are regarded as classical confounding ***patient factors*** in most epidemiological studies, whereas tumor thickness according to Breslow, the presence or absence of ulceration, invasion level according to Clark, and histological type are common ***disease-specific confounding factors***.

During recent years, the use of **directed acyclic graphs** (**DAGs**) has become an increasingly popular alternative method to evaluate confounders in a complex setting. In a DAG, exposure, outcome and one or several other variables are connected by one-directed nodes and edges that do not form cycles. These factors can then be evaluated in a systematic fashion to determine the need of adjustment(s). DAGs were used together with conventional evaluation of confounders for all studies (***I-IV***) of this thesis.

In ***studies I and III***, data on sex, age, tumor thickness according to Breslow, the presence or absence of ulceration, and invasion level according to Clark were collected as potential confounders of the investigated association between tumor site and patient survival. Sex and age only were considered as confounders of the association between tumor site and sentinel node location (***study I***), and between tumor site and incidence trends (***study II***).

Interaction occurs when the joint effect of two variables is greater than the individual effects put together. The net effect can be synergistic, antagonistic, additive or multiplicative. In

study I, we evaluated possible multiplicative interactions between sex, age and calendar period, and in ***study III*** between detailed anatomic site and visibility of skin site.

4.6 DATA SOURCES

Medical records

The foundation of all studies (***I−IV***) was a medical record review for detailed body site of the primary tumor. For ***study I***, the review also included information on sentinel node location and status.

SkinTrac©

Site was registered and body division models created in the software SkinTrac© (previously ***EssDoll***©), a three-dimensional anatomic model of the human body developed for detailed site classification of the body surface by our group in the late 1990s (***studies I−IV)*** (121).

The precision of recorded tumors was classified on a five-level scale [***0−2.5 cm***, ***2.5−5 cm, 5-10 cm***, ***10-15 cm***, ***or > 15 cm*** (previously "estimate")] according to the maximum radius of uncertainty. The two best precision categories were equivalent to site information obtained from photographies, sketches or detailed text descriptions such as "5 cm above the left areola" or "right zygomatic bone", whereas the third and fourth categories represented less precise descriptions such as "left popliteal fossa" and "right pectoral muscle", respectively. The least precise category was used for descriptions such as "upper back". If site information was available only from the ICD code (and not covered by the five-level scale), detailed body site was classified as missing in the present thesis. The latter differs slightly from the work by *Gillgren et al* (116), where such tumors were registered on the most common site among tumors registered within the three best precision categories for the anatomic area covered by the relevant ICD code.

Registers

The population-based **Regional Melanoma Register of Stockholm and Gotland** (RMR) (118) holds records of patients diagnosed with melanoma since 1976, and is the source of inclusion for studies ***II−IV***. Data is prospectively reported by physicians in accordance with a care program ("Vårdprogram"), and includes various clinical and histopathological parameters such as date of diagnosis, ICD code and established confounders. For studies ***II−IV***, all patient and tumor variables except detailed body site of the primary tumor were derived from the RMR. The coverage of the register is high, with a mean coverage of more than 98% for studies II and III.

The Swedish Melanoma Register (SMR) (122) was founded in 2003 to unite data from the regional melanoma registers, and the included variables are thus largely the same as for the RMR. The register was used in ***study I*** for inclusion, patient and tumor variables similarly to studies II−IV.

The **Cause-of-Death Register** (123) is a national register that collects causes of death for deceased Swedish residents. The register is held by the National Board of Health and Welfare and data, used in ***studies I and III,*** include both the underlying and contributing causes of death classified by ICD codes.

The **Total Population Register** is a national register held by Statistics Sweden, and the source of the emigration data in ***studies I and III*** (124)**. Statistics Sweden** also compiles a broad range of official national and regional population statistics such as the size of the population by calendar year, sex and age (***study II***) (125).

Mutation analyses

New analyses of *NRAS* and *BRAF* mutation status were determined by standard (*NRAS*) or nested (*BRAF*) polymerase chain reaction (***PCR***) [B] and **pyrosequencing** [C] (***study IV***) (126,127). Previously published included analyses had been performed using the same method, complemented by (65), or only analyzed by (128), standard or seminested PCR followed by single strand conformational polymorphism (***SSCP***) and ***nucleotide sequence analysis*** [D].

[B] PCR is a method to copy a DNA (deoxyribonucleic acid) segment, where a DNA template containing the segment of interest, together with complementary oligonucleotides (primers), a DNA polymerase enzyme and nucleotides under the influence of thermic cycles generate a chain reaction of exponential DNA replication. ***Nested PCR*** is a two-step (outer and inner PCR) method to optimize correct binding. In ***study IV***, the regions around *NRAS* exon 2 codon 61 and *BRAF* exon 15 codon 600 underwent PCR for amplification.

[C] Pyrosequencing was named by the pyrophosphate release when a complementary nucleotide is added to a base of a single strand DNA during DNA synthesis. Given that nucleotides are added one by one (A, T, C and G) sequentially and that the release of pyrophosphate (through ATP and enzyme mediated reactions) emits light that is proportional to the amount of nucleotide incorporation, the DNA sequence of the single strand DNA can be visually determined as peaks on a ***pyrogram***.

[D] The form (conformation) of a single strand DNA sequence changes (polymorphism) if a base changes. This phenomenon can be seen as changed electrophoretic mobility and is utilized by SSCP to detect mutations. Since SSCP only detects the presence or absence of mutations, mutated samples need to undergo subsequent nucleotide sequence analysis to determine the specific genotype.

4.7 STATISTICAL METHODS

Statistical methods are applied to make inference about true population estimates from sample data.

Nonparametric tests

To test differences in the distribution of categorical variables, we used the **Fisher's exact test** [E] for basic variables (***studies I–IV)***, and the **chi squared test** [F] for main variables (***study IV)***. To describe differences in the distribution of basic numerical variables, we used the **Wilcoxon rank-sum test** (Mann-Whitney U test) [G] (***studies I–IV)*.**

[E] The Fisher's exact test calculates the exact probability of observed counts using factorials:

$$p = \frac{(a+b)! * (c+d)! * (a+c)! * (b+d)!}{n! * a! * b! * c! * d!}$$

a, b, c, d = observed frequency in cells a, b, c and d, respectively
n = sum of frequencies a + b + c + d

[F] The chi squared test is based on the (squared) difference between observed and expected counts:

$$X^2 = \sum_{i=1}^{n_i} \sum_{j=1}^{n_j} \frac{\left(O_{ij} - E_{ij}\right)^2}{E_{ij}}$$

n_i = number of cells in row i
n_j = number of cells in column j
O_{ij} = observed frequency in cell ij
E_{ij} = expected frequency in cell ij

[G] The Wilcoxon rank-sum test is based on the difference between median values and compares the sum of ranks. The formula for large samples is displayed below:

$$W^* = \frac{W - n_s(n_s + n_L + 1)/2}{\sqrt{\frac{n_s n_L (n_s + n_L + 1)}{12}}}$$

W = sum of ranks for smaller sample
n_s = sample size of the group with the smaller sum of ranks
n_s = sample size of the group with the larger sum of ranks

Regression models

Regression models are used to investigate the association between one or several independent (explanatory) exposure variables and a dependent (response/outcome) variable and are reported using various measures of **relative risk** (RR). ***Univariate (crude)*** regression models investigate one independent variable in relation to a dependent variable and return the combined association of the independent variable and possible confounders, whereas ***multivariate*** regression models include several independent variables and return the confounder-***adjusted*** direct association between an independent and dependent variable of interest.

In ***study 1***, we used **logistic regression model**s [H] to determine the association between detailed tumor site and sentinel node locations, and **Cox proportional hazards regression model**s [I] to determine the association between the variables above and patient survival. The proportional hazards assumption was visually examined using a scatter plot of scaled Schoenfeld residuals over time. Cox proportional hazards regression was also used in the survival analyses of ***study III***. In ***study III***, the main analyses were complemented by **competing risk regression model**s [J] to provide estimates in the presence of competing causes of death. In ***study II***, we used **Poisson regression model**s [K] to evaluate incidence trends of melanoma.

[H] ***Odds*** is a risk measure of the probability (Pr) of experiencing an event versus the probability of not experiencing that event:

$$Odds = \frac{Pr}{1 - Pr}$$

Odds ratio (OR) is used to compare the odds of two groups (exposed and unexposed):

$$OR = \frac{Odds_{exposed}}{Odds_{unexposed}}$$

Logistic regression is suitable when the dependent variable is dichotomous. It is estimated as an OR. The underlying odds function of exposed and unexposed is calculated as follows:

$$Log(odds) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n$$

x = independent variables
β = coefficient for x

[I] ***Cox proportional hazards regression*** estimates as a hazard ratio (HR), where the hazard represents the risk of experiencing an event of interest at a specific time, assuming survival up until that time:

$$H(t) = H_0(t) * exp(\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n)$$

$H(t)$ = hazard at time t
$H_0(t)$ = hazard at time t when all variables x = 0
x = independent variables
β = coefficient for x

If the hazards in the nominator and denominator are proportional, the HR is constant over time:

$$HR = \frac{H_{exposed}(t)}{H_{unexposed}(t)} = exp(\beta_{exposed})$$

[J] ***Competing risk regression*** according to Fine and Gray returns estimates for an event of interest (death due to melanoma), given that a competing event (death due to other causes) has not occurred. It is based on the subdistribution hazard function (in contrast to the cause-specific hazard function of the Cox proportional hazards regression):

$$H^*(t) = \lim_{\Delta t \to 0} \frac{Pr\{t \leq T < t + \Delta t, \varepsilon = k \mid T \geq t \cup (T \leq t \cap \varepsilon \neq k)\}}{\Delta t}$$

$H^*(t)$ = subdistribution hazard at time t
Pr = probability
T = event time
ε = event type
k = event of interest

[K] ***Incidence proportion*** (IP, also commonly referred to as cumulative incidence) is a risk measure of the proportion of new cases during a specific time period in a population:

$$IP = \frac{n_{new\ cases}}{n_{at\ risk}}$$

To account for the rate with which new cases develop, ***incidence rate*** (IR), is preferred:

$$IR = \frac{n_{new\ cases}}{\sum(pt_{at\ risk})}$$

pt = person-time

Incidence rate ratio (IRR) is used to compare the IR of two groups (exposed and unexposed):

$$IRR = \frac{IR_{exposed}}{IR_{unexposed}}$$

Poisson regression can be modeled as a rate-based regression, where the log rate denominator (person-time) is handled as an offset. The regression estimate is a rate ratio (incidence rate ratio), where the underlying rate function (incidence rate) of exposed and unexposed is calculated as follows:

$$Log(IR) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n$$

x = independent variables
β = coefficient for x

Age-standardized incidence rates

Age-standardized incidence rates by the direct method were used in ***study II*** to estimate incidence rates of melanoma over time in a (theoretical) setting where the age distribution of the underlying population is constant over time, thus removing the effect of age as a confounder. This is achieved by weighting the crude incidence rates to the age distribution of a standard population for whom the age distribution is known. We chose the Swedish 1979 population as standard. The age-standardized incidence rate can be calculated as follows:

$$IR_{st} = \sum_i d_i w_i / y_i$$

IR_{st} = age-standardized incidence rate
d_i = number of cases in age group i
w_i = weight of age group i
y_i = person-time in age group i

Tests of interaction

In ***studies II and III***, we performed tests of interaction using the ***multiplicative*** method [L], where interaction is indicated if, for the underlying RR function:

[L] $\beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \neq \beta_1 x_1 + \beta_2 x_2$

$\beta_{12} x_1 x_2$ = interaction term

Sensitivity analyses

Sensitivity analyses are performed to test the robustness (sensitivity) of the obtained results to altered features of the primary analyses. In ***study III***, sensitivity analyses were performed by using a broader definition of melanoma-specific death (including both underlying and contributing causes of death), and a stricter definition of UVR exposure site (excluding acral lentiginous melanoma).

5 RESULTS

5.1 STUDY I

Out of the 859 individuals with melanoma, 495 had melanoma located on the trunk and 394 on the extremities. 66% of individuals with trunk melanoma were men, whereas those with extremity melanoma were mainly women (63%). 54% of trunk melanomas were located on the upper back. The presence of ulceration (37% vs 29%) or more than one positive sentinel node in the same sentinel node location (23% vs. 16%) was higher among trunk melanomas than extremity melanomas.

Sentinel node location

Multiple (31% vs. 7%) and contralateral (25% vs. 1%) sentinel node locations were more common among individuals with trunk melanoma, compared to extremity melanoma. Further, having multiple sentinel node locations correlated visually with a detailed anatomic site near the midline of the body (*see original paper Suppl. figure 2*).

Uncommon sentinel node locations occurred with a similar frequency (8% vs. 7%) for individuals with trunk and extremity melanoma, including intrathoracal or -abdominal locations (3% vs 4%). Intraabdominal or -thoracal sentinel node locations among extremity melanomas were predominantly related to deep pelvic nodes originating from lower extremity melanomas (n = 13), whereas only one intrathoracal node location was observed among upper extremity melanomas.

In multivariable sex- and age-adjusted models, trunk melanoma (compared to extremity melanoma) was strongly and independently associated with the presence of multiple (adjusted odds ratio (aOR) 7.1; 95% CI 4.6–11.5) and contralateral (aOR 32.2; 95% CI 15.1–83.7) sentinel node locations, but not with uncommon sentinel node locations (aOR 1.1; 95% CI 0.6–1.9) (**Table 5.1.1**).

Survival

The median follow-up time was 4.9 years. During the study period, 147 individuals (21% of trunk melanomas; 12% of extremity melanomas) died from melanoma, and 45 (5% of trunk melanomas; 5% of extremity melanomas) from other causes. Three emigrated, whereas the rest were followed alive to the study endpoint.

The adjusted hazard ratio (aHR) of melanoma-specific death was 1.9 (95% CI 1.3–2.9) for patients with trunk melanoma, compared to extremity melanoma. The risk was highest for individuals with tumors on the upper back (aHR 2.3, CI 1.4–3.6) and borderline higher (aHR 2.0, CI 1.0–3.8) on the lower front, whereas those with tumors on the lower back and upper

front of the trunk showed no prognostic differences compared to the patients with melanoma on the extremities (**Table 5.1.2**).

Patients with multiple, contralateral, uncommon or inaccessible sentinel node locations were not at increased risk of melanoma-specific death, compared to patients without these features (**Table 5.1.3**). It should be noted, though, that the number of events in the two last groups (*i.e.* uncommon and inaccessible) was low.

Table 5.1.1. Adjusted odds ratios of multiple, contralateral or uncommon sentinel node locations by detailed body site of cutaneous melanoma.

Detailed site	Sentinel node location					
	Multiple		Contralateral		Uncommon	
	aOR[a]	95% CI[b]	aOR	95% CI	aOR	95% CI
Extremity	1.0	Ref[c]	1.0	Ref	1.0	Ref
Trunk	**7.1**	**4.6–11.5**	**32.2**	**15.1–83.7**	1.1	0.6–1.9

[a] Adjusted odds ratio. Adjustments for sex and age.

[b] Confidence interval

[c] Reference category

Table 5.1.2. Adjusted hazard ratios of melanoma-specific death by detailed body site of cutaneous melanoma.

Detailed site	At risk (n)	Events (%)	aHR[a]	95% CI
Extremity	393	13	1.0	Ref
Upper back	251	24	**2.3**	**1.4–3.6**
Lower back	67	13	1.0	0.5–2.4
Upper front	79	19	1.6	0.9–3.0
Lower front	68	21	**2.0**	**1.0–3.8**

[a] Adjusted hazard ratio. Adjustments for sex, age, tumor thickness, ulceration, Clark invasion level, sentinel node status, uncommon, multiple and contralateral sentinel node location.

Table 5.1.3. Adjusted hazard ratios of melanoma-specific death by sentinel node locations of cutaneous melanoma.

Sentinel node location	At risk (n)	Events (%)	aHR[a]	95% CI
No			1.0	Ref
Multiple (yes)	171	19	1.1	0.4–2.9
Contralateral (yes)	118	25	1.0	0.4–2.5
Uncommon (yes)	66	11	0.5	0.2–1.4

[a] Adjustments for sex, age, tumor thickness, ulceration, invasion level, sentinel node status, other sentinel node sites, and melanoma site.

5.2 STUDY II

The cohort consisted of 3,058 individuals diagnosed with melanoma during five time periods: 1977−1978 (n = 321), 1983−1984 (n = 466), 1989−1990 (n = 730), 1995−1996 (n = 703) and 2000−2001 (n = 838). Information on detailed anatomic site of the primary tumor was available in 93% of cases. Divided by ultraviolet radiation exposure (UVR) exposure site, 1,653 had melanoma on highly intermittent† sites, 620 on moderately intermittent‡ sites, and 391 on chronic sites.

Individuals with melanoma on highly and moderately intermittent UVR exposure sites were on average younger (median age 56 / 59 years; interquartile range (IQR) 43−68 / 44−73) than those with melanoma on chronic UVR exposure sites (median age 71 years; IQR 59−79). Individuals with melanoma on highly intermittent UVR exposure sites were predominantly (60%) men, whereas those with melanoma on moderately intermittent UVR exposure sites were mostly (78%) women. Among individuals with melanoma on chronic UVR exposure sites, the difference in distribution between the sexes was smaller (45% men; 55% women).

† Referred to as *intermittent (core)* sites in the original manuscript.

‡ Referred to as *intermittent (peripheral)* sites in the original manuscript.

Age-standardized incidence rates

Using the Swedish 1979 population as standard, the age-standardized incidence rate (IR) of melanoma increased on intermittent UVR exposure sites both among men (7.8 to 16.5 cases/10^5 person-years) and women (7.6 to 14.6 cases/10^5 person-years) from the first time period to the last. The corresponding IR of melanoma on chronic UVR exposure sites increased from 1.7 to 2.3 cases/10^5 person-years among men, and from 1.4 to 1.8 cases/10^5 person-years among women.

Poisson regression

Adjusted for sex and age, the overall risk of melanoma on intermittent sites doubled over time (adjusted rate ratio (aRR) 2.1, 95% CI 1.8−2.4), with similar estimates for melanoma on highly intermittent (RR 2.1, 95% CI 1.8−2.5) and moderately intermittent (RR 2.1, 95% CI 1.6−2.7) sites. Meanwhile, the adjusted relative risk of melanoma on chronic UVR exposure sites increased with 50% (aRR 1.4 (95% CI 1.0−1.9) (**Tables 5.2.1−5.2.2)**.

Sex and age

The incidence increase of melanoma on intermittent UVR exposure sites was most pronounced in older age groups (> 65 years of age), especially among men ($p < 0.0001$). Meanwhile, the incidence increase on chronic UVR sites was stable (*i.e.* no significant interactions were detected) across different age groups and between the sexes.

The relative risk of developing melanoma on highly intermittent UVR exposure sites was higher for men than for women (aRR 1.7, 95% CI 1.5−1.9), whereas the opposite was the case for moderately intermittent UVR exposure sites (aRR 0.3; 95% CI 0.3−0.4). For chronic UVR exposure sites, the risk of developing melanoma was only associated with advanced age (aRR 5.3, 95% CI 4.1−6.8 compared to the youngest age group).

Invasivity

Subgroup analyses revealed that invasive melanomas on intermittent UVR exposure sites increased over time (aRR 1.9, 95% CI 1.6−2.2) like the estimates of the overall data in this group, whereas those at chronic UVR exposure sites did not (aRR 1.0, 95% CI 0.7−1.4). *In situ* melanomas increased at both intermittent (adjusted RR 5.8, 95% CI 3.6−10.0) and chronic (adjusted RR 4.0, 95% CI 2.0−9.2) UVR exposure sites.

Table 5.2.1. Adjusted rate ratios of cutaneous melanoma at intermittent (overall) and chronic ultraviolet radiation (UVR) exposure sites by calendar period.

Calendar period	UVR exposure site			
	Intermittent (overall)		Chronic	
	aRR[a]	95% CI[b]	aRR	95% CI
1977−1978	1.0	Ref[c]	1.0	Ref
1983−1984	1.5	1.2–1.7	1.0	0.7–1.4
1989−1990	1.9	1.6–2.2	1.3	0.9–1.8
1995−1996	1.7	1.4–1.9	1.4	1.0–1.9
2000−2001	**2.1**	**1.8–2.4**	**1.4**	**1.0–1.9**

[a] Adjusted rate ratio. Adjustments for sex and age.

[b] Confidence interval

[c] Reference category

Table 5.2.2. Adjusted rate ratios of cutaneous melanoma at highly intermittent and moderately intermittent ultraviolet radiation (UVR) exposure sites by calendar period.

Calendar period	UVR exposure site			
	Highly intermittent		Moderately intermittent	
	aRR[a]	95% CI	aRR	95% CI
1977−1978	1.0	Ref	1.0	Ref
1983−1984	1.4	1.2–1.7	1.5	1.1–2.0
1989−1990	2.1	1.8–2.5	1.4	1.1–1.9
1995−1996	1.7	1.4–2.1	1.5	1.2–2.1
2000−2001	**2.1**	**1.8–2.5**	**2.1**	**1.6–2.7**

[a] Adjustments for sex and age

5.3 STUDY III

A total of 5,973 melanoma patients were analyzed. Of these, 52% were women and 48% were men. The distribution of melanoma sites differed between the sexes. Among men, 61% were trunk melanomas, whereas among women, trunk melanomas were the second most common category (33%), preceded by leg melanomas (39%). The site of the primary melanoma also differed by age of diagnosis, with a median age of 70 years among individuals diagnosed with head-neck melanoma, and 55 years among those with melanoma on the legs.

The median follow-up was 14 years. Of the 2,919 individuals who did not survive the entire follow-up period, 906 (31%) died from melanoma and the rest from other causes. 125 patients were lost to follow-up due to emigration.

UVR exposure model

Classified by UVR exposure site, melanomas on highly intermittent UVR exposure sites were more common (60%) than those on moderately intermittent (23%) or chronic (13%) UVR exposure sites. Further, melanomas on highly intermittent UVR exposure sites displayed more favorable tumor characteristics (thickness, ulceration and invasion level), but a higher proportion of melanoma-specific death (16%) than those on moderately intermittent (13%) and chronic (12%) UVR exposure sites.

With regard to patient characteristics, individuals with melanoma on highly intermittent UVR exposure sites were dominated by men (58%) and melanoma on moderately intermittent sites by women (76%), whereas those with melanoma on chronic UVR exposure sites displayed a more even sex distribution (women 52%, men 48%). The age at diagnosis was lower among individuals with highly intermittent (median 55 years) than moderately (59 years) or chronic (72 years) UVR exposure sites.

The adjusted hazard ratio (aHR) of melanoma-specific death was lower among individuals with melanoma on moderately intermittent (aHR 0.7; CI 0.6−0.8) and chronic (aHR 0.6; CI 0.4−0.7) UVR exposure sites than among those with melanoma on highly intermittent UVR exposure sites (**Table 5.3.1**). Sensitivity analyses using a broader definition of melanoma-specific death (underlying *or* contributing cause of death) provided similar results for intermittent and chronic UVR exposure sites. Stratified analyses by sex also revealed similar associations. UVR exposure site was not associated with overall survival, which is not surprising given the long follow-up and large proportion of other-cause deaths (69% of all deaths).

Skin visibility model

Poor visibility of the primary melanoma site upon skin self-examination was associated with a higher relative risk of melanoma-specific death than easily visible sites even after multivariable adjustment for established confounders (aHR 1.3; CI 1.1−1.5) (**Table 5.3.2**). There was no significant interaction between the UVR exposure and skin visibility models.

Anatomic subsite model

Individuals with trunk melanoma were at increased risk of melanoma-specific death compared to those with melanoma on the arms, legs, head-neck, and acral sites (**Table 5.3.3**). Subdivision of the trunk, arms and legs did not improve prognostic prediction.

Among head-neck and acral sites, the neck, and palms and soles were associated with a higher relative risk of melanoma-specific death than the face and ears, and subungual sites, respectively (**Table 5.3.4**). However, the event numbers in these categories were rather small.

Table 5.3.1. Adjusted hazard ratios of melanoma-specific death by ultraviolet radiation (UVR) exposure site.

UVR exposure site	At risk	Events	Partial model		Full model	
	n	%	a_pHR[a]	95% CI[b]	a_fHR[c]	95% CI
Highly intermittent	3,600	16	1.0	Ref[d]	1.0	Ref
Moderately intermittent	1,371	13	0.9	0.7–1.0	0.7	0.6–0.8
Chronic	609	12	0.7	0.5–0.9	**0.6**	**0.4–0.7**

[a] Partially adjusted hazard ratio. Adjustments for sex, age and year of diagnosis.

[b] Fully adjusted hazard ratio. Adjustments for sex, age, year of diagnosis, tumor thickness, ulceration, and Clark invasion level.

[c] Confidence interval

[d] Reference category

Table 5.3.2. Adjusted hazard ratios of melanoma-specific death by skin visibility upon skin self-examination.

UVR exposure site	At risk	Events	Partial model		Full model	
	n	%	a_pHR	95% CI	a_fHR	95% CI
Easily visible	3,311	13	1.0	Ref	1.0	Ref
Poorly visible	2,662	18	1.3	1.2–1.5	**1.3**	**1.1–1.5**

Table 5.3.3. Adjusted hazard ratios of melanoma-specific death by major anatomic site.

Major anatomic sites	At risk	Events	Partial model		Full model	
	n	%	a_pHR	95% CI	a_fHR	95% CI
Trunk	2,887	18	1.0	Ref	1.0	Ref
Arms	800	11	0.6	0.5–0.7	0.5	0.4–0.6
Legs	1,497	12	0.7	0.6–0.9	0.6	0.5–0.7
Head-neck	686	14	0.7	0.6–0.9	0.6	0.5–0.8
Acral	103	27	1.5	1.0–2.1	0.6	0.4–1.0

Table 5.3.4. Adjusted hazard ratios of melanoma-specific death by anatomic subsite.

Anatomic subsites	At risk	Events	Partial model		Full model	
	n	%	a_pHR	95% CI	a_fHR	95% CI
Head-neck						
Face and ears	549	12	1.0	Ref[d]	1.0	Ref
Scalp	44	27	2.6	1.4–4.8	1.2	0.7–2.3
Neck	93	19	1.7	1.0–2.8	1.8	1.0–3.0
Acral						
Palms and soles	68	29	1.0	Ref	1.0	Ref
Combined subungual area	35	23	0.7	0.3–1.5	0.2	0.2–1.0

5.4 STUDY IV

Of the eligible new samples, DNA extraction/PCR was successful for 139 samples (94%). Of these, *NRAS* mutation status was determined for all samples, and *BRAF* mutation status for 91%. Adding the 185 previously studied samples, the final cohort consisted of 324 samples.

145 samples originated from melanomas on chronic UVR exposure sites, and 164 from melanomas on intermittent UVR exposure sites. Individuals with melanoma on chronic UVR exposure sites were older than those with melanoma on intermittent UVR exposure sites (median 75 vs. 62 years). Further, melanomas on chronic UVR exposure sites were thinner (median 1.6 vs. 2.6 mm), less invasive (33% vs. 1% *in situ*) and less ulcerated (19% vs. 45%) than those on intermittent UVR exposure sites. The most common histological type was lentigo maligna melanoma (LMM, 47%) on chronic UVR exposure sites, and superficial spreading melanoma (SSM, 50%) on intermittent UVR exposure sites.

Mutation status

The overall proportion of *BRAF* and *NRAS* mutated tumors was lower on chronic UVR exposure sites than on intermittent UVR exposure sites. *BRAF* mutations were more common than *NRAS* mutations in samples originating from melanomas on intermittent UVR exposure sites (49% vs. 28%), whereas *NRAS* mutations were more common than *BRAF* mutations (17% vs. 10%) in samples originating from melanomas on chronic UVR exposure sites. The mutations were mutually exclusive except for one sample. Using the χ^2 test, *BRAF* mutations were associated with intermittent UVR exposure sites, and *NRAS* mutations with chronic UVR exposure sites (p-value < 0.0001) (**Table 5.4.1**).

Table 5.4.1. x^2 test of mutation status[a] by UVR exposure site.

Mutation status	UVR exposure site		
	Intermittent	**Chronic**	**p-value**
BRAF mutated	81	13	< 0.0001
NRAS mutated	46	22	
Wild type	35	96	

[a] Not including 15 tumors with undetermined *BRAF* status, and 1 with both *BRAF* and *NRAS* mutations

6 DISCUSSION

CAUSALITY

All studies (***I–IV***) in this thesis measure associations and not causality. This implies that a statistical association between factor A and factor B does not (necessarily) mean that factor B is caused by factor A; it simply implies that the two factors relate to each other. Causality on the other hand measures whether factor B is, or is not, an effect of factor A. These differences need to be kept in mind when interpreting the results. The evidence obtained from well-performed cohort studies is preceded only by controlled trials.

6.1 INTERNAL VALIDITY

Internal validity can be explained as the ability of a study to measure what it intended to measure, *i.e.* (causal) associations. This ability is crucial to all scientific studies, and a high internal validity is characterized by a lack of ***bias*** as well as unadjusted ***confounding***. It is also of interest to have a low probability of ***chance*** findings.

6.1.1 Bias

Bias is a ***systematic error*** in the design of a study that may create spurious associations between the exposure and outcome(s) of interest, either removing true associations or evoking (positive or negative) associations that are in reality not there. The feature that makes bias especially problematic compared to confounding is that its effect cannot be removed or adjusted for in statistical analyses. Neither can it be reduced by increasing the study sample. Hence, avoiding or minimizing bias is a main concern already at the design phase in all studies.

The nomenclature to describe different types of bias is extensive and not very consistent. However, bias can be roughly divided into two categories, selection bias and information bias, based on underlying mechanisms.

Selection bias occurs when the individuals in the study sample differ from the source population with regard to the risk of being exposed or developing the outcome of interest. **Information bias** (also commonly referred to as ***observation bias***) instead refers to misclassification or measurement error. Information bias can be ***dependent*** (differential) or ***independent*** (non-differential) of the exposure of interest, and affect ***exposure*** as well as ***outcome(s***) and confounders.

Study-specific aspects

The population-based design of ***studies I–III*** reduces the risk of selection bias in these studies, whereas the tissue samples analyzed in ***study IV*** may be at greater risk of this type of bias.

As for information bias, there is a possibility of misclassification of exposure, *i.e.* detailed anatomic site of the primary cutaneous melanoma in ***all studies*** (***I–IV***) due to the retrospective review of medical records and uncertainty of recordings. Further, the determination of UVR exposure pattern is based on presumed exposure by body site and not on actual exposure data such as individual sun habits and protective measures. Neither can we rule out the possibility of misclassification of outcome(s), represented by sentinel node location and status (***study I***), causes of death (***studies I*** and ***III***) and mutation status (***study IV***). Established confounders are less likely to be misclassified, given the prospective registration and high validity of these patient and tumor characteristics in the regional and national quality registers.

To reduce the risk of misclassification of detailed anatomic site (***studies I–IV***), we put large efforts into the data collection. This is reflected in the low uncertainty (an estimated radius of <5 cm) in the majority of site recordings.

The main concern with regard to the laboratory analyses in ***study IV*** was the possibility of measurement error, *i.e.* the possibility of false classification of tissue samples as wild type in the presence of mutations. To reduce this risk, we applied established laboratory methods (*see section 4.6*). However, it should be kept in mind that the sensitivity of these methods is not 100% and a differentially misclassified component cannot be excluded.

The accuracy of the reported causes of death in the Cause-of-Death Register is strengthened by the fact that death certificates are provided by the treating physicians, but may be inaccurately captured in some cases. Also, the distinction between underlying and contributing causes of death may be equivocal. In the main analyses of this thesis, we classified death as melanoma-specific only when melanoma was registered as the underlying cause of death, thereby increasing the positive predictive value of the classification (***studies I*** and ***III***). We also re-computed all analyses using all-cause death as outcome.

The fact that the estimates in ***study I*** changed only marginally during the five-year median follow-up in analyses of overall compared to melanoma-specific death indicates that most deaths within this time period were melanoma-specific, and that a potential degree of false-negative misclassification of melanoma-specific deaths would hardly have influenced the results at all. Meanwhile, the lack of differences in overall survival in ***study III*** is not surprising given the long median follow-up (14 years), entailing a large number of other-cause deaths among both exposed and unexposed that dominated these analyses.

In ***study III***, we also added a sensitivity analysis where we changed the definition of melanoma-specific death to include both underlying and contributing causes of death listing

melanoma. The reason for this was that individuals diagnosed with melanoma on chronic UVR exposure sites were on average older and thus at a higher risk of dying from other causes than melanoma, which may reduce the likelihood of melanoma being accurately classified as the underlying cause of death.

6.1.2 Confounding

Confounding (*see section 4.5*) is the second major threat to the internal validity of a study. However, confounders may be controlled for in analyses through statistical **adjustment**, **stratification** or **restriction** of the confounding variable, hence removing the effect of this variable from the (causal) association under investigation.

In this thesis, we used multivariable regression analysis to enable simultaneous adjustment of potential and established confounders (***studies I*** and ***III***). However, there are still several pitfalls related to adjustment. First, confounding variables must be carefully assessed and only true confounders included to avoid ***overadjustment*** (129). Second, all true confounders need to be included and properly quantified to avoid ***residual confounding***.

Study-specific aspects

To avoid overadjustment and minimize the risk of residual confounding, we pre-assessed the exposure(s), outcome(s) and established as well as potential confounders using directed acyclic graphs (DAGs) (*see section 4.5*) for ***all studies*** (***I–IV***). Further, we modeled continuous variables linearly (***studies I*** and ***III***) or using splines (for non-linear variables) (***study III***) instead of categorization when applicable.

The fact that sex, age and established tumor factors such as thickness, the presence or absence of ulceration and Clark invasion level are prospectively recorded in the RMR and SMR facilitated proper adjustment for these factors. However, there may be some residual confounding due to unidentified or unmeasured confounders. These include comorbidities, socioeconomic and cohabitation status with regard to patient survival (***studies I*** and ***III***) and sentinel node status (***study III***). For ***study I***, information on sentinel node status was collected as part of the medical record review.

6.1.3 Chance

Lastly, there is the possibility of chance findings, often referred to as ***random error***. The probability of chance findings is estimated using p-values and/or confidence intervals. Whereas ***p-values*** only assess the probability of finding an estimate of the same, or a more extreme, magnitude by chance only, ***confidence intervals*** quantify this uncertainty by providing an interval that with a pre-set probability contains the true population estimate, while also indirectly providing the information of a p-value. Hence, confidence intervals are more informative when evaluating statistical associations.

The p-value and confidence interval limits are constructed cut-off levels to determine whether associations are ***statistically significant***. The significance (alfa) level is often set to 5% (0.05) for p-values, corresponding to a 95% confidence interval, but can be altered to achieve increased precision.

The possibility of chance findings can never be eliminated, but the risk is reduced with increasing sample size and number of outcome events.

Study-specific aspects

In ***all studies*** (***I–IV***), the significance level was pre-set to 0.05. In ***studies I–III***, the main estimates of association were reported together with confidence intervals (with or without p-values), whereas p-values only were calculated for ***study IV***. Further, the sample sizes were larger, and the risk of chance findings hence lower, than in most previous studies.

6.2 EXTERNAL VALIDITY

External validity refers to the extent to which the conclusions of a study are ***generalizable*** to populations other than the one studied. External validity is often high for large observational studies, especially population-based studies, since the study population and the source population are similar (given that the internal validity is also high). This is also the reason why well-performed high-internal validity RCTs may be at risk of low external validity; the selection of study participants may generate conclusions that are true only under a strict set of criteria.

Study-specific aspects

The generalizability of studies ***I–III*** is high due to the population-based design and an estimated high internal validity. In ***study IV***, the latter is more difficult to determine. Further, this thesis only investigates cutaneous melanoma. Conclusions thus cannot be applied on non-cutaneous melanoma (mucosal melanoma, ocular melanoma).

6.3 FINDINGS AND IMPLICATIONS

6.3.1 Study I

Sentinel node location

We found that cutaneous melanoma located on the trunk was associated with multiple and contralateral but not uncommon sentinel node locations, compared to melanoma located on the extremities.

The prevalence of multiple sentinel node locations in trunk melanoma was in the upper range of previous studies (106–112) and, as hypothesized, much higher than that of extremity sites.

However, the fact that we did not find any differences in the prevalence of uncommon sentinel node locations between trunk and extremity sites was against our a priori hypothesis, and influenced by an unexpectedly high frequency of deep pelvic nodes among extremity melanomas. The observed preponderance of multiple sentinel node locations near the midline of the body was an interesting side finding, although for anatomic reasons maybe not very surprising.

Survival

In the survival analyses, we confirmed that individuals with cutaneous melanoma located on the trunk had a reduced melanoma-specific survival compared to those with melanoma on the extremities. This was especially evident for melanoma subsites on the upper back. Multiple, contralateral or uncommon sentinel node locations were however not associated with survival.

There are not many studies on detailed anatomic trunk site and patient survival and those that exist are not consistent (116,130–135). Hence, the fact that we found prognostic differences within the trunk area, and that the subsite of interest overlaps with the BANS (upper back, posterior arm, neck and scalp) (130) and TANS (thorax (back and breast), upper arm, neck and scalp) (136) areas was interesting.

The lack of association between multiple sentinel node locations and patient survival is in line with two smaller (109,112) and one larger (110) previous study, but not with another smaller (108) study. In other words, evidence is still not clear but increasingly points toward multiple sentinel node locations as unrelated to prognosis.

There are even fewer studies on uncommon sentinel node locations and patient survival (98,99), perhaps due to the rareness of these events. Further, the definition of uncommon sentinel node location differs between studies, complicating comparisons. Still, our results together with a larger study of interval nodes support the lack of association with survival for uncommon sentinel node locations, although inference in our study was somewhat limited due to low event numbers.

It should also be noted that inaccessible nodes (*see section 4.4*) were more common among uncommon than common sentinel node locations, and that the evaluation of uncommon sentinel node locations and survival included adjustment for these nodes. Although inaccessible nodes were not associated with a reduced survival in our study, the HR and confidence interval tended toward the latter (aHR 1.9, 95% CI 0.8−4.2 compared to verified negative nodal status). It is feasible to hypothesize that this may reflect the presence of positive as well as negative nodes among these unexplored inaccessible nodes. The lack of surgical node exploration was in fact a major limitation of a smaller study (99) of patients with in-transit sentinel node locations that showed opposite results.

Strengths

Strengths of the study include the population-based design, the detailed anatomic site description, and the use of prospectively collected quality register data and high-coverage national register data for confounders and causes of death/losses to follow-up (emigration), respectively.

Limitations

Limitations include limited power to detect smaller prognostic differences, potential misclassification of detailed anatomic site due to the retrospective study design, potential underestimation of (uncommon) SNLs in the lymphatic mapping procedure and/or documentation of the latter, and potential misclassification of causes of death (*see section 6.1.1*).

6.3.2 Study II

Incidence increase

We found that site-assigned intermittent UVR exposure was a driver of the incidence increase of cutaneous melanoma, whereas site-assigned chronic UVR exposure appeared to be of less importance. The incidence increase of melanoma on intermittent UVR exposure sites was influenced by age- and sex-specific factors, whereas that of chronic UVR exposure sites was not.

Although causality is not applicable, these findings support the hypothesis of intermittent UVR exposure (15–25) as a main risk factor behind the incidence increase of cutaneous melanoma. The presence of sex-specific risk patterns and interactions between calendar period, age and sex on these sites may reflect influence from behavioral aspects (137) such as clothing, sun habits and protective measures, although biological factors (138,139) may also contribute to site differences between the sexes. Meanwhile, the absence of the features above but a clear association with advanced age implies that chronic UVR exposure is largely related to cumulative sun-induced damage, which may be less prone to changes over time.

Analyses of invasive and *in situ* melanomas separately supported that a component of diagnostic drift (2) and/or improved early detection may have taken place during the study period. However, the overall conclusions were not affected by this.

Another interesting observation, which was not in line with our a priori hypothesis, was that we could not detect any differences in relative incidence increase between highly intermittent and moderately intermittent sites. It is unclear to which degree the experimental nature of this categorization may have influenced these results.

Possible biological mechanisms behind the stronger incidence increase detected among melanomas on intermittent than chronic UVR exposure sites include a preponderance of

sunburn (19–24,26–34) on these sites, whereas the development of a gradual tan from chronic UVR exposure has been suggested as protective factor against sunburn.

Strengths

Strengths include (like above) the population-based design, the detailed anatomic site description, and the use of quality register as well as high-coverage national register data.

Limitations

Limitations chiefly concern the lack of actual individual UVR exposure data. Other limitations include the lack of data for the past 15 years, during which age and sex-specific sun behavior as well as diagnostic criteria may have changed.

Still, it may be argued that the use of site as a proxy variable for UVR exposure patterns is likely to be at lower risk of self-selection and recall bias, and a less subjective measure of exposure than that obtained from self-assessment in interview/questionnaire studies. Further, proper randomization of UVR exposure is difficult, and well-performed population-based large observational studies may often be the best level of evidence available.

Given the difficulties involved in evaluating individual UVR exposure patterns over a lifetime, there is a need for different approaches to measure this exposure. Hence, the classification of presumed UVR exposure based on detailed anatomic site may, together with other studies of different limitations, contribute to important knowledge with regard to primary prevention.

6.3.3 Study III

UVR exposure model

We found a lower melanoma-specific survival among individuals diagnosed with melanoma on highly intermittent UVR exposure sites than among those with melanoma on chronic or moderately intermittent UVR exposure sites, independently of differences in established patient and tumor factors or skin visibility upon self-examination (SSE).

These results are interesting given that the few previous studies addressing this research question have shown no (140), or a borderline protective (141,142), association between intermittent UVR exposure and melanoma-specific survival. These previous questionnaire or interview studies were not (fully) adjusted for tumor factors, and all but one (140) were smaller than ours. Further, the definitions and measures of intermittent UVR exposure varied between the studies and some may not have fully captured the exposure.

The results for chronic UVR exposure sites are in line with previous studies of markers of cumulative sun-induced damage and patient survival (141,143).

Whereas increased levels of vitamin D, nitric oxide (144), melanin and DNA damage-repair (141,145) have been suggested as plausible protective UVR-related mechanisms behind favorable outcomes, the underlying biology of a possible association between intermittent UVR exposure and an unfavorable outcome has been less elaborated. Since *BRAF* mutations have been separately linked to sunburn (41), primary location on the trunk (37,62–65), skin areas with no or low signs of cumulative sun-induced damage (37–40,62), and (variably to) reduced survival (146), it would be interesting to look further into *BRAF* mutations in this context.

Skin visibility model

The reduced survival we found among individuals with melanoma on poorly, compared to easily, visible skin sites upon SSE even after adjustments of established prognostic factors was an unexpected finding, as was the lack of significant interactions with UVR exposure site. Although differences in UVR patterns may partially explain the results, other biological mechanisms may be involved and increased attention to these sites is warranted.

Anatomic subsite model

Although we detected prognostic differences upon subdivision of head-neck and acral sites, these findings were limited by low event numbers. The most interesting finding from the anatomic subsite model, and against our a priori hypothesis, was instead the lack of statistically significant prognostic differences within the trunk and extremities. These results contrast with the findings from ***study I*** as well as those of a previous study from our group (116), although the studies are not directly comparable due to different categorizations and statistical approaches.

The detailed categorization used may contribute to a limited power for individual subsites, and we cannot exclude the possibility that we might have been able to detect prognostic differences by combining subsites into larger areas or by increasing the sample size. For example, we noted that melanoma-specific death among individuals with trunk melanoma was more common for melanoma subsites on the thorax and back (subsites no. 1−4; 17.1−19.8%) than on the abdomen and lower trunk (subsites no. 5−7; 13.4−14.5%). On the other hand, this is to our knowledge the largest study performed on detailed anatomic site and patient survival, and it may be sufficient to conclude that a detailed subdivision of the trunk and extremities does not appear to add any large prognostic value.

Strengths

As for ***studies I*** and ***II***, strengths include the population-based design, the detailed anatomic site description, the use of quality register as well as high-coverage national register data. The study is also of appreciable size, especially considering that the assessment of exposure is based on medical record reviews.

Limitations

Limitations are also largely shared by ***studies I*** and/or ***II***, and include limited power to detect smaller prognostic differences, potential misclassification of detailed anatomic site due to the retrospective study design, inherent limitations from the lack of individual actual UVR exposure data and use of detailed site as a proxy variable of the latter, lack of adjustment for sentinel node status and potential other confounding from comorbidities, socioeconomic (83) and cohabitation (84) status, and potential misclassification of causes of death (*see section 6.1.1*).

6.3.4 Study IV

We confirmed our hypothesis that *BRAF* mutations were associated with melanoma on intermittent UVR exposure sites, and *NRAS* mutations with melanoma on chronic UVR exposure sites. Further, we observed that the overall prevalence of *BRAF* and *NRAS* mutations among melanomas on chronic compared to intermittent UVR exposure sites was low.

This has previously been implicated in studies where intermittent and chronic UVR exposure were defined by major anatomic sites and/or the absence or presence of signs of chronic sun-induced damage (37–40,62–65) but is now demonstrated in a large cohort using an established UVR exposure model. Although causality cannot be determined, the results support that *NRAS* and *BRAF* mutations are related to different UVR exposure patterns, but also imply that other genetic mechanisms (40,147,148) may be more relevant with regard to chronic UVR exposure and melanoma development.

An association between *BRAF* mutations and intermittent UVR exposure is biologically supported by a demonstrated increased incidence of melanoma among *BRAF* V600E mutated mice (compared to non-V600E *BRAF* mice) after UVR exposure resulting in sunburn (41).

Strengths

Strengths include the detailed anatomic site description, the use of quality register data and robust methods for mutation screening, and the large sample size.

Limitations

Although it has been demonstrated that *NRAS* and *BRAF* mutations are early events and that detected mutations in metastatic tissue can be extrapolated to the primary lesion, the use of primary tumor tissue samples in the new cohort vs. metastatic tissue in the majority of samples in the previously studied cohort may constitute a limitation of the study, as may the use of partially different screening methods [pyrosequencing (*new*) vs. pyrosequencing/SSCP and nucleotide sequence analysis (*prev. studied*)] and sample storage methods [formaline-fixed paraffine-embedded (FFPE; *new*) vs. fresh frozen/FFPE (*prev. studied*)] between the new and previously studied cohort, and the long storage time for the majority of the samples.

7 CONCLUSIONS

Summary conclusion

The main conclusions of this thesis are that:

- The lower survival among patients with trunk melanoma (compared to the extremities) is not related to uncommon or multiple sentinel node locations (***study I***).
- Site-assigned intermittent UVR exposure appears more relevant than chronic UVR exposure for the incidence increase of cutaneous melanoma (***study II***).
- Site-assigned intermittent UVR exposure is a negative prognostic factor compared to chronic UVR exposure (***study III***).
- Site-assigned intermittent UVR exposure is related to *BRAF* mutations, and chronic UVR exposure to *NRAS* mutations (***study IV***).

Specific conclusions

Study I

- Trunk melanoma is related to multiple sentinel node locations, with a visual preponderance near the midline of the body.
- Trunk melanoma is related to increased risk of melanoma-specific death, especially if located on the upper back.
- Trunk melanoma is not related to uncommon sentinel node locations.
- Multiple and uncommon sentinel node locations are not related to increased risk of melanoma-specific death.

Study II

- Melanoma located on intermittent UVR exposure sites increased more over time than melanoma on chronic UVR exposure sites.
- The incidence increase of melanoma on intermittent UVR exposure sites was most pronounced in older age groups (> 65 years of age), especially among men.
- The incidence increase on chronic UVR sites was stable across different age groups and between the sexes.

Study III

- Melanoma located on highly intermittent UVR exposure sites are related to a lower patient survival than moderately intermittent and chronic UVR exposure sites.
- Melanoma on poorly visible sites upon skin self-examination are also related to a lower patient survival than easily visible sites.
- Subdivision of the major anatomic sites trunk, arms and legs is not clinically relevant with regard to patient survival.

Study IV

- Intermittent UVR exposure sites are related to *BRAF* mutations, and chronic UVR exposure sites to *NRAS* mutations in melanoma.
- *BRAF* and *NRAS* mutations are less prevalent on chronic UVR exposure sites than on intermittent UVR exposure sites.

8 FUTURE PERSPECTIVES

In Australia, decades of systematic UVR protection approaches have resulted in a decline in the incidence of melanoma among adolescents and young adults (149). However, whereas the UV index in Australia is constantly high or very high, the Swedish UV index is predominantly low with only short periods of high levels, and successful strategies in Australia cannot be assumed to have similar impact in a high-latitude setting.

Given the continued incidence increase of melanoma in Sweden, there is an imperative incentive to re-evaluate the primary prevention efforts of melanoma used so far. To achieve this, large-scale studies, such as nationwide pre-post intervention trials, and cluster randomization of kindergartens, child health centers and outdoor sport clubs, are needed.

The interventions should be coordinated by a single institution or authority, with a multi-professional team of physicians, epidemiologists, statisticians, economists, administrators, educators and public relations professionals, and collaborations with dermatological and oncological organizations.

In addition to conventional information by authorities and healthcare, the public should be actively approached from multiple sources with a focus on social media, TV, radio and newspaper advertising.

There should be multiple research questions/interventions. For example, it would be interesting to evaluate the impact of appearance-related interventions and the use of UV index and sun protection advice through mobile apps such as “Min soltid” by the Swedish Radiation Authority (150) among young people, and the impact of UV index guided sun protective measures (shadow, clothing, sunscreen) by kindergarten teachers and sport club leaders, and parent education by pediatric nurses. An important intervention by the Swedish, Finnish, Norwegian and Icelandic Radiation Authorities (151) to follow, is the prohibition against the use of solar beds for cosmetic purposes for children and adolescents that will take place in 2018.

Short- as well as long-term outcomes should be evaluated. Short-term outcomes of interest include different measures of UV exposure as well as the prevalence of nevi and erythema (markers of intermittent UVR exposure and sunburn), whereas long-term outcomes include the incidence of melanoma. It would also be interesting to look at melanoma-specific, cardiovascular and overall survival in this context. Nationwide register-based outcomes should be combined with in-depth surveys on sample cohorts.

This would naturally demand a sizeable long-term funding. However, it is important not to forget that the costs of primary prevention should be evaluated in relation to the costs related to melanoma on a societal level (6). Of these, there are not only direct costs related to healthcare, but also indirect costs from productivity losses.

The perspectives above are not novel; several measures are in fact already covered by the Swedish Radiation Authority (152). Many aspects were also discussed during the Vadstena 3 meeting “Facts and consensus about skin cancer prevention” (Svenska Dermato-Epidemiologiska Nätverket (SveDEN) and Svenska Sällskapet för Dermatologisk Kirurgi och Onkologi (SDKO), 2016).

9 SAMMANFATTNING PÅ SVENSKA

Bakgrund: Samspelet mellan primär tumörlokalisation, exponering för ultraviolett (UV) strålning, genetiska faktorer, insjuknande i och överlevnad vid malignt melanom är komplext. I denna avhandling syftade vi att undersöka effekterna av detaljerad tumörlokalisation bortom den konventionella och strikt anatomiska uppdelningen i huvud-hals, bål, övre och nedre extremiteter, med fokus på indelning av kroppsytan enligt UV-exponeringsmönster i förhållande till dessa faktorer.

Metoder: Vi inhämtade och registrerade detaljerad information avseende primärtumörens lokalisation i ett skräddarsytt datorprogram, och klassificerade (beroende på studie) kroppsytor enligt dominerande UV-exponeringsmönster, synlighet för ögat vid självundersökning av huden, samt anatomiska uppdelningar mer detaljerade än ICD-klassifikationen (International Classification of Diseases) (***studie I–IV)***. Genom länkning till regionala och/eller nationella register erhöll vi (beroende på studie) uppgifter om insjuknande och överlevnad såväl som etablerade störfaktorer och (i förekommande fall) bortfall. Vi inhämtade även information avseende lokalisation och status för portvaktskörtel/-körtlar (***studie I***), och utförde PCR och pyrosekvensning för detektion av mutationer i proto-onkogenerna *BRAF* och *NRAS* (***studie IV***).

Resultat: Melanom på bålen var associerat med förekomst av multipla men inte ovanliga sentinel node-lokalisationer jämfört med melanom på extremiteter. Multipla eller ovanliga lokalisationer av portvaktskörtel/-körtlar kunde inte identifieras som störfaktorer i förhållandet mellan melanom på bålen och reducerad patientöverlevnad (***studie I***). Melanom med intermittenta UV-exponeringsmönster, klassificerade enligt detaljerad anatomisk lokalisation av den primära tumören, var associerade med en högre incidensökning från 1970- till 2000-talet i Stockholm-Gotlandsregionen jämfört med lokalisationer med kroniskt UV-exponeringsmönster (***studie II***). Anatomiska lokalisationer med höggradigt intermittenta UV-exponeringsmönster och lokalisationer med låg synlighet för ögat vid självundersökning av huden var associerade med minskad patientöverlevnad, jämfört med kroniskt UV-exponerade och väl synliga lokalisationer (***studie III***). Intermittent UV-exponerade lokalisationer var associerade med *BRAF*-mutationer, och kroniskt UV-exponerade lokalisationer med *NRAS*-mutationer (***studie IV***).

Slutsats: Primära tumörlokalisationer med intermittenta UV-exponeringsmönster förefaller relaterade till incidensökningen såväl som minskad patientöverlevnad och genetiska *BRAF*-mutationer vid hudmelanom, medan lokalisationer som tilldelas kroniska UVR-exponeringsmönster bidrar mindre till incidensökningen, är prognostiskt mer fördelaktiga och övervägande uppvisar *NRAS*-mutationer. Multipla eller ovanliga lokalisationer av portvaktskörtel/-körtlar förklarar inte den sämre överlevnaden hos patienter med melanom på bålen.

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